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     FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
     13:30:20 ON 27 DEC 2001
L1         2568 S 2.3.1.41 OR (KETOACYL (W) (SYNTHETASE OR SYNTHASE)) OR KAS OR
L2          467 S L1 AND (MUTANT# OR MUTATION# OR MUTAGENESIS)
L3          188 DUP REM L2 (279 DUPLICATES REMOVED)
L4          142 S L3 NOT PY>1999
L5           57 S L4 AND (COLI OR PROKARY? OR PROCARY? OR PLANT)
L6            4 S L5 AND SUBSTRATE
L7           17 S L4 AND SPECIFICITY

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N):y

L5 ANSWER 1 OF 57 MEDLINE
ACCESSION NUMBER: 1999444087 MEDLINE
DOCUMENT NUMBER: 99444087 PubMed ID: 10512619
TITLE: Conversion of a beta-**ketoacyl synthase** to a malonyl decarboxylase by replacement of the active-site cysteine with glutamine.
AUTHOR: Witkowski A; Joshi A K; Lindqvist Y; Smith S
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, California 94609, USA.
CONTRACT NUMBER: DK 16073 (NIDDK)
SOURCE: BIOCHEMISTRY, (1999 Sep 7) 38 (36) 11643-50.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991101
Last Updated on STN: 20000303
Entered Medline: 19991021

AB beta-Ketoacyl synthases involved in the biosynthesis of fatty acids and polyketides exhibit extensive sequence similarity and share a common reaction mechanism, in which the carbanion participating in the condensation reaction is generated by decarboxylation of a malonyl or methylmalonyl moiety; normally, the decarboxylation step does not take place readily unless an acyl moiety is positioned on the active-site cysteine residue in readiness for the ensuing condensation reaction. Replacement of the cysteine nucleophile (Cys-161) with glutamine, in the beta-**ketoacyl synthase** domain of the multifunctional animal fatty acid synthase, completely inhibits the condensation reaction but increases the uncoupled rate of malonyl decarboxylation by more than 2 orders of magnitude. On the other hand, replacement with Ser, Ala, Asn, Gly, and Thr compromises the condensation reaction without having any marked effect on the decarboxylation reaction. The affinity of the beta-**ketoacyl synthase** for malonyl moieties, in the absence of acetyl moieties, is significantly increased in the Cys161Gln **mutant** compared to that in the wild type and is similar to that exhibited by the wild-type beta-**ketoacyl synthase** in the presence of an acetyl primer. These results, together with modeling studies of the Cys --> Gln **mutant** from the crystal structure of the Escherichia **coli** beta-**ketoacyl synthase** II enzyme, suggest that the side chain carbonyl group of the Gln-161 can mimic the carbonyl of the acyl moiety in the acyl-enzyme intermediate so that the **mutant** adopts a conformation analogous to that of the acyl-enzyme intermediate. Catalysis of the decarboxylation of malonyl-CoA requires the dimeric form of the Cys161Gln fatty acid synthase and involves prior transfer of the malonyl moiety from the CoA ester to the acyl carrier protein domain and subsequent release of the acetyl product by transfer back to a CoA acceptor. These results suggest that the role of the Cys --> Gln beta-ketoacyl synthases found in the loading domains of some modular polyketide synthases likely is to act as malonyl, or methylmalonyl, decarboxylases that provide a source of primer for the chain extension reactions catalyzed by associated modules containing fully competent beta-ketoacyl synthases.

L5 ANSWER 2 OF 57 MEDLINE
ACCESSION NUMBER: 1999264304 MEDLINE
DOCUMENT NUMBER: 99264304 PubMed ID: 10330468
TITLE: CUT1, an Arabidopsis gene required for cuticular wax biosynthesis and pollen fertility, encodes a very-long-chain fatty acid **condensing enzyme**.
AUTHOR: Millar A A; Clemens S; Zachgo S; Giblin E M; Taylor D C; Kunst L
CORPORATE SOURCE: Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada.
SOURCE: PLANT CELL, (1999 May) 11 (5) 825-38.
Journal code: BJU; 9208688. ISSN: 1040-4651.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF129511
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990730
Last Updated on STN: 20000303
Entered Medline: 19990722

AB Land plants secrete a layer of wax onto their aerial surfaces that is essential for survival in a terrestrial environment. This wax is composed of long-chain, aliphatic hydrocarbons derived from very-long-chain fatty

acids (VLCFAs). Using the Arabidopsis expressed sequence tag database, we have identified a gene, designated CUT1, that encodes a VLCFA **condensing enzyme** required for cuticular wax production. Sense suppression of CUT1 in transgenic Arabidopsis plants results in waxless (eceriferum) stems and siliques as well as conditional male sterility. Scanning electron microscopy revealed that this was a severe waxless phenotype, because stems of CUT1-suppressed plants were completely devoid of wax crystals. Furthermore, chemical analyses of waxless plants demonstrated that the stem wax load was reduced to 6 to 7% of wild-type levels. This value is lower than that reported for any of the known eceriferum **mutants**. The severe waxless phenotype resulted from the downregulation of both the decarbonylation and acyl reduction wax biosynthetic pathways. This result indicates that CUT1 is involved in the production of VLCFA precursors used for the synthesis of all stem wax components in Arabidopsis. In CUT1-suppressed plants, the C24 chain-length wax components predominate, suggesting that CUT1 is required for elongation of C24 VLCFAs. The unique wax composition of CUT1-suppressed plants together with the fact that the location of CUT1 on the genetic map did not coincide with any of the known ECERIFERUM loci suggest that we have identified a novel gene involved in wax biosynthesis. CUT1 is currently the only known gene with a clearly established function in wax production.

L5 ANSWER 3 OF 57 MEDLINE
ACCESSION NUMBER: 1999174280 MEDLINE
DOCUMENT NUMBER: 99174280 PubMed ID: 10074711
TITLE: KCS1 encodes a fatty acid elongase 3-ketoacyl-CoA synthase affecting wax biosynthesis in Arabidopsis thaliana.
AUTHOR: Todd J; Post-Beittenmiller D; Jaworski J G
CORPORATE SOURCE: Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA.
SOURCE: PLANT JOURNAL, (1999 Jan) 17 (2) 119-30.
Journal code: BRU; 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990426
Last Updated on STN: 19990426
Entered Medline: 19990413
AB An Arabidopsis fatty acid elongase gene, KCS1, with a high degree of sequence identity to FAE1, encodes a 3-ketoacyl-CoA synthase which is involved in very long chain fatty acid synthesis in vegetative tissues, and which also plays a role in wax biosynthesis. Sequence analysis of KCS1 predicted that this synthase was anchored to a membrane by two adjacent N-terminal, membrane-spanning domains. Analysis of a T-DNA tagged kcs1-1 **mutant** demonstrated the involvement of the KCS1 in wax biosynthesis. Phenotypic changes in the kcs1-1 **mutant** included thinner stems and less resistance to low humidity stress at a young age. Complete loss of KCS1 expression resulted in decreases of up to 80% in the levels of C26 to C30 wax alcohols and aldehydes, but much smaller effects were observed on the major wax components, i.e. the C29 alkanes and C29 ketones on leaves, stems and siliques. In no case did the loss of KCS1 expression result in complete loss of any individual wax component or significantly decrease the total wax load. This indicated that there was redundancy in the elongase KCS activities involved in wax synthesis. Furthermore, since alcohol, aldehyde, alkane and ketone levels were affected to varying degrees, involvement of the KCS1 synthase in both the decarbonylation and acyl-reduction wax synthesis pathways was demonstrated.

L5 ANSWER 4 OF 57 MEDLINE
ACCESSION NUMBER: 1999170152 MEDLINE
DOCUMENT NUMBER: 99170152 PubMed ID: 10071936
TITLE: Kinetics and equilibrium studies of Tet repressor-operator interaction.
AUTHOR: Kedracka-Krok S; Wasylewski Z
CORPORATE SOURCE: Physical Biochemistry Department, Institute of Molecular Biology, Jagiellonian University, Krakow, Poland.
SOURCE: JOURNAL OF PROTEIN CHEMISTRY, (1999 Jan) 18 (1) 117-25.
Journal code: AEJ; 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990607
Last Updated on STN: 19990607
Entered Medline: 19990527
AB Binding of a Tet repressor **mutant** containing a single Trp43 residue in the tet operator recognition alpha-helix leads to the quenching

of the protein fluorescence down to about 23% in the case of the tet O1 operator and to 40% in the case of the tet O2 operator. We have used fluorescence detection to describe the binding equilibrium and kinetics of the Tet repressor interaction with the 20-bp DNA operators tet O1 and tet O2. Stopped-flow measurements in an excess of the tet operators performed in 5 mM NaCl or 150 mM NaCl indicate that the reaction can be described by at least three exponentials characterized by different relaxation times. The mechanism of interaction for both operators as well as for two salt concentrations used can be described as TetR + Operator <==> Complex 1 <==> Complex 2 <==> Complex 3. Only the much faster process can be described as a second-order reaction characterized by a bimolecular rate constant equal to 2.8X10(6) M(-1) sec(-1) for both operators. The medium and slow processes may be described by relaxational times ranging from 50 msec to seconds. The results of the binding equilibrium measurements extrapolated to 1 M NaCl concentration, which reflects the specific nonionic interaction between TetR and tet operators, indicate **Kas** equal to 3.2x10(4) and 4.0x10(5) M(-1) for tet O1 and tet O2, respectively. The number of monovalent ions replaced upon binding can be calculated as about 5 and 3 for tet O1 and tet O2, respectively. The binding of Tet repressor to the operators leads to changes in the circular dichroism spectra of the DNA which could indicate transitions of B-DNA into A-like DNA structure.

L5 ANSWER 5 OF 57 MEDLINE
ACCESSION NUMBER: 1998345981 MEDLINE
DOCUMENT NUMBER: 98345981 PubMed ID: 9681003
TITLE: Condensing enzymes from Cuphea wrightii associated with medium chain fatty acid biosynthesis.
AUTHOR: Slabaugh M B; Leonard J M; Knapp S J
CORPORATE SOURCE: Department of Crop and Soil Science, Oregon State University, Corvallis 97331, USA.. slabaugm@css.orst.edu
SOURCE: PLANT JOURNAL, (1998 Mar) 13 (5) 611-20.
Journal code: BRU; 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U67316; GENBANK-U67317
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980817

AB Seed oils of most Cuphea species contain > 90% medium chain (C8-C14) fatty acids. Thioesterases with specificity for these substrates are important determinants of the medium chain phenotype. The role of condensing enzymes, however, has not been investigated. cDNA clones encoding beta-**ketoacyl-acyl carrier protein** (ACP) synthase (**KAS**) were isolated from C. wrightii, a C10/C12-producing species. Deduced amino acid sequences of four unique clones were approximately 60% identical to **plant KAS** I sequences and approximately 75% identical to a distinct class of **KAS** sequences recently identified in castor and barley. A 46 kDa protein that was observed only in developing and mature seed was detected using antiserum directed against recombinant Cuphea **KAS** protein. The 46 kDa protein was abundant in developing seeds of six medium chain-producing Cuphea species but barely detected in one long chain-producing species. A 48 kDa protein identified immunologically as **KAS** I was expressed in both medium and long chain-producing Cuphea species and was detected in all tissues tested. In in vitro assays, extracts from C. wrightii and C. viscosissima developing embryos were unable to extend fatty acid chains beyond C10 following treatment with 10 microns cerulenin, a potent inhibitor of **KAS** I. However, a C. viscosissima **mutant**, cpr-1, whose seed oils are deficient in caprate relative to wild type, was impaired in extension of C8 to C10 in this assay and Western analysis revealed a specific deficiency in 46 kDa **KAS** in cpr-1 embryos. These results implicate cerulenin-resistant condensing activity in production of medium chain fatty acids in Cuphea.

L5 ANSWER 6 OF 57 MEDLINE
ACCESSION NUMBER: 1998153133 MEDLINE
DOCUMENT NUMBER: 98153133 PubMed ID: 9485400
TITLE: The malonyl/acetyltransferase and beta-**ketoacyl synthase** domains of the animal fatty acid synthase can cooperate with the acyl carrier protein domain of either subunit.
AUTHOR: Joshi A K; Witkowski A; Smith S
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, 747 Fifty-Second Street, Oakland, California 9460.
CONTRACT NUMBER: DK 16073 (NIDDK)
SOURCE: BIOCHEMISTRY, (1998 Feb 24) 37 (8) 2515-23.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980407
Last Updated on STN: 19980407
Entered Medline: 19980320

AB The active form of the animal fatty acid synthase (FAS) is a dimer of identical multifunctional polypeptides, each containing seven discrete functional domains, that cooperate to form two centers for palmitate synthesis. To assess the importance of domain cooperation across the subunit interface in the reaction mechanism, we have utilized a strategy based on complementation analysis in vitro of modified FASs carrying critical **mutations** in specific catalytic domains. Homodimeric FASs carrying the same **mutation**(s) in both subunits are unable to synthesize fatty acids. As predicted by the current head-to-tail model for the animal FAS, heterodimeric FASs formed between the acyl carrier protein (ACP) **mutant** and either the beta-**ketoacyl synthase** (KS) or malonyl/acetyltransferase (MAT) are active in palmitate synthesis, confirming that the KS and MAT domains can cooperate with the ACP domain of the opposite subunit. Contrary to this model however, heterodimeric FASs formed between the KS and MAT **mutants**, between a MAT, ACP double **mutant**, and a KS **mutant**, and between a KS, ACP double **mutant**, and a MAT **mutant** are also active in palmitate synthesis, indicating that the MAT and KS domains can also cooperate with the ACP domain of the same subunit. The results of this study reveal an unanticipated element of redundancy in the FAS reaction mechanism in that the amino-terminal KS and MAT domains can make functional contact with the penultimate carboxy-terminal ACP domain of either subunit. A revised model for the FAS is proposed in which the substrate loading and condensation reactions can be catalyzed either by one of the two subunits or by cooperation between domains across the subunit interface.

L5 ANSWER 7 OF 57 MEDLINE
ACCESSION NUMBER: 1998070260 MEDLINE
DOCUMENT NUMBER: 98070260 PubMed ID: 9405069
TITLE: Characterization of the interthiol acyltransferase reaction catalyzed by the beta-**ketoacyl synthase** domain of the animal fatty acid synthase.
AUTHOR: Witkowski A; Joshi A K; Smith S
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, California 94609, USA.
CONTRACT NUMBER: DK 16073 (NIDDK)
SOURCE: BIOCHEMISTRY, (1997 Dec 23) 36 (51) 16338-44.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980130
Last Updated on STN: 20000303
Entered Medline: 19980122

AB The enzyme activity responsible for translocation of saturated acyl chains from the 4'-phosphopantetheine of the acyl carrier protein to the active site cysteine of the beta-**ketoacyl synthase** in the animal fatty acid synthase has been identified. An enzyme assay was devised that allows uncoupling of the interthiol transfer step from the condensation reaction. Experiments with various fatty acid synthase **mutants** indicate clearly that catalysis of the transfer of saturated acyl moieties from the 4'-phosphopantetheine thiol to the active site cysteine thiol, Cys-161, is an inherent property of the beta-**ketoacyl synthase** domain. Catalytic efficiency of the interthiol transferase increases from C2 to C12 and decreases with increasing chain-lengths beyond C12. Malonyl, beta-hydroxybutyryl, and crotonyl thioesters are not substrates for the transferase, whereas the beta-ketobutyryl moiety is a poor substrate. These features of the substrate specificity are exactly as predicted for a transferase that fulfills the proposed role in the fatty acid synthase reaction sequence and indicate that this activity plays an important role in determining the overall specificity of the beta-**ketoacyl synthase** reaction.

L5 ANSWER 8 OF 57 MEDLINE
ACCESSION NUMBER: 97431479 MEDLINE
DOCUMENT NUMBER: 97431479 PubMed ID: 9286984
TITLE: Fatty acid biosynthesis in Pseudomonas aeruginosa: cloning and characterization of the fabAB operon encoding beta-hydroxyacyl-acyl carrier protein dehydratase (FabA) and beta-**ketoacyl-acyl carrier protein** synthase I (FabB).

AUTHOR: Hoang T T; Schweizer H P
CORPORATE SOURCE: Department of Microbiology, Colorado State University, Fort Collins 80523, USA.
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Sep) 179 (17) 5326-32.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U70470
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19971013
Last Updated on STN: 19980206
Entered Medline: 19970930

AB The Pseudomonas aeruginosa fabA and fabB genes, encoding beta-hydroxyacyl-acyl carrier protein dehydratase and beta-**ketoacyl-acyl carrier protein** synthase I, respectively, were cloned, sequenced, and expressed in Escherichia **coli**. Northern analysis demonstrated that fabA and fabB are cotranscribed and most probably form a fabAB operon. The FabA and FabB proteins were similar in size and amino acid composition to their counterparts from Escherichia **coli** and to the putative homologs from Haemophilus influenzae. Chromosomal fabA and fabB **mutants** were isolated; the **mutants** were auxotrophic for unsaturated fatty acids. A temperature-sensitive fabA **mutant** was obtained by site-directed **mutagenesis** of a single base that induced a G101D change; this **mutant** grew normally at 30 degrees C but not at 42 degrees C, unless the growth medium was supplemented with oleate. By physical and genetic mapping, the fabAB genes were localized between 3.45 and 3.6 Mbp on the 5.9-Mbp chromosome, which corresponds to the 58- to 59.5-min region of the genetic map.

L5 ANSWER 9 OF 57 MEDLINE
ACCESSION NUMBER: 97227931 MEDLINE
DOCUMENT NUMBER: 97227931 PubMed ID: 9119048
TITLE: Amounts of proteins altered by **mutations** in the dnaA gene of Escherichia **coli**.
AUTHOR: Ohba A; Mizushima T; Katayama T; Sekimizu K
CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka, Japan.
SOURCE: FEBS LETTERS, (1997 Mar 10) 404 (2-3) 125-8.
Journal code: EUH; 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970506
Last Updated on STN: 19980206
Entered Medline: 19970418

AB We identified proteins whose amounts were altered in a temperature-sensitive dnaA46 **mutant** of Escherichia **coli** . Proteins whose amounts were increased in the **mutant** were serine hydroxymethyltransferase, beta-**ketoacyl** [**acyl carrier protein**] synthase II, long-chain fatty acid transport protein, and UDP-glucose 4-epimerase, while the decreased ones were flagellin and D-ribose-binding protein. Transformation of the **mutant** with a plasmid containing the wild type dnaA gene complemented the phenotype. As pulse-labeling experiments revealed that the rates of synthesis of the proteins were altered in the **mutant** , DnaA protein may be involved in expression of these proteins.

L5 ANSWER 10 OF 57 MEDLINE
ACCESSION NUMBER: 96326321 MEDLINE
DOCUMENT NUMBER: 96326321 PubMed ID: 8759840
TITLE: Bacillus subtilis acyl carrier protein is encoded in a cluster of lipid biosynthesis genes.
AUTHOR: Morbidoni H R; de Mendoza D; Cronan J E Jr
CORPORATE SOURCE: Department of Microbiology, University of Illinois, Urbana 61801, USA.
CONTRACT NUMBER: AI15650 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1996 Aug) 178 (16) 4794-800.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U59433
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19961008
Last Updated on STN: 19980206
Entered Medline: 19960920

AB A cluster of Bacillus subtilis fatty acid synthetic genes was isolated by complementation of an Escherichia **coli** fabD **mutant** encoding a thermosensitive malonyl coenzyme A-acyl carrier protein transacylase. The B. subtilis genomic segment contains genes that encode three fatty acid synthetic proteins, malonyl coenzyme A-acyl carrier protein transacylase (fabD), 3-**ketoacyl-acyl carrier protein** reductase (fabG), and the N-terminal 14 amino acid residues of acyl carrier protein (acpP). Also present is a sequence that encodes a homolog of E. **coli** plsX, a gene that plays a poorly understood role in phospholipid synthesis. The B. subtilis plsX gene weakly complemented an E. **coli** plsX **mutant**. The order of genes in the cluster is plsX fabD fabG acpP, the same order found in E. **coli**, except that in E. **coli** the fabH gene lies between plsX and fabD. The absence of fabH in the B. subtilis cluster is consistent with the different fatty acid compositions of the two organisms. The amino acid sequence of B. subtilis acyl carrier protein was obtained by sequencing the purified protein, and the sequence obtained strongly resembled that of E. **coli** acyl carrier protein, except that most of the protein retained the initiating methionine residue. The B. subtilis fab cluster was mapped to the 135 to 145 degrees region of the chromosome.

L5 ANSWER 11 OF 57 MEDLINE
ACCESSION NUMBER: 96279127 MEDLINE
DOCUMENT NUMBER: 96279127 PubMed ID: 8663183
TITLE: An isoleucine to valine substitution in Escherichia **coli** acyl carrier protein results in a functional protein of decreased molecular radius at elevated pH.
AUTHOR: Keating D H; Cronan J E Jr
CORPORATE SOURCE: Department of Microbiology, University of Illinois, Urbana-Champaign, Urbana, Illinois 61801, USA.
CONTRACT NUMBER: AI15650 (NIAID)
RR07141 (NCRR)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Jul 5) 271 (27) 15905-10.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960911
Last Updated on STN: 19970203
Entered Medline: 19960829

AB Escherichia **coli** acyl carrier protein (ACP) has been reported to exist in at least two distinct conformers in solution. A novel form of ACP having an increased electrophoretic mobility on polyacrylamide gel electrophoresis was noted previously during work on beta-**ketoacyl -acyl carrier protein** synthase II (fabF) **mutants** of E. **coli** (Jackowski, S., and Rock, C. O.(1987) J. Bacteriol. 169, 1469-1473). These workers reported that the increased electrophoretic mobility of the ACP from fabF strains occurred irrespective of prosthetic group attachment or the state of acylation of the prosthetic group. Since these workers were unable to detect a difference between the amino acid sequence of the ACP from the fabF **mutants** and that of wild type ACP, they suggested that the increased electrophoretic mobility was due to an unknown post-translational modification of the polypeptide chain. We have reinvestigated these **mutants** and report that the increased electrophoretic mobility is due to a **mutation** within the gene (acpP) that encodes ACP. This **mutation** results in substitution of isoleucine for valine 43 of ACP. Site-directed **mutagenesis** of a synthetic ACP gene demonstrated that the amino acid substitution at residue 43 is the cause of the increased electrophoretic mobility. Gel filtration experiments indicated that the increased electrophoretic mobility results from the more compact structure of V43I ACP at high pH. The altered residue lies within the ACP region of greatest conformational lability, and thus the V43I substitution may shift the equilibrium toward the more compact conformation(s). The disulfide-linked dimer of V43I ACP was readily formed and had an electrophoretic migration greater than the dimer of wild type ACP, suggesting that formation of ACP.ACP dimers does not require structural deformation of the protein.

L5 ANSWER 12 OF 57 MEDLINE
ACCESSION NUMBER: 96086932 MEDLINE
DOCUMENT NUMBER: 96086932 PubMed ID: 7500937
TITLE: Structural analysis of PKS1, a polyketide synthase gene involved in melanin biosynthesis in Colletotrichum lagenarium.
AUTHOR: Takano Y; Kubo Y; Shimizu K; Mise K; Okuno T; Furusawa I
CORPORATE SOURCE: Laboratory of Plant Pathology, Faculty of Agriculture, Kyoto University, Japan.

SOURCE: MOLECULAR AND GENERAL GENETICS, (1995 Nov 15) 249 (2) 162-7.
Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D83643
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 19990129
Entered Medline: 19960117

AB Albino **mutants** (Pks-) of Colletotrichum lagenarium form nonmelanized appressoria and possess little penetrating ability on the host **plant**. The defect in albino **mutant** 79215 (Pks-) is considered to lie in pentaketide biosynthesis and/or pentaketide cyclization during melanin biosynthesis. The cosmid pAC7, carrying the PKS1 gene, when transformed into the albino **mutant** restores the wild-type melanin phenotype. We have determine the DNA sequence and the transcriptional organization of the PKS1 gene. The PKS1 gene contains one open reading frame, consisting of 3 exons separated by two short introns. The predicted PKS1 polypeptide consists of 2187 amino acids and shows significant similarities with other polyketide synthases, particularly that encoded by wA in Aspergillus nidulans, involved in conidial pigmentation. The PKS1 gene contains highly conserved beta-**ketoacyl synthase**, acetyl/malonyl transferase, and acyl carrier protein domains. We propose that the C. lagenarium PKS1 gene encodes a polyketide synthase involved in melanin biosynthesis.

L5 ANSWER 13 OF 57 MEDLINE
ACCESSION NUMBER: 96064736 MEDLINE
DOCUMENT NUMBER: 96064736 PubMed ID: 7592873
TITLE: Enoyl-acyl carrier protein reductase (fabI) plays a determinant role in completing cycles of fatty acid elongation in Escherichia **coli**.
AUTHOR: Heath R J; Rock C O
CORPORATE SOURCE: Department of Biochemistry, St Jude Children's Research Hospital, Memphis, Tennessee 38101, USA.
CONTRACT NUMBER: CA 21765 (NCI)
GM34496 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 3) 270 (44) 26538-42.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951221

AB The role of enoyl-acyl carrier protein (ACP) reductase (E.C. 1.3.1.9), the product of the fabI gene, was investigated in the type II, dissociated, fatty acid synthase system of Escherichia **coli**. All of the proteins required to catalyze one cycle of fatty acid synthesis from acetyl-CoA plus malonyl-CoA to butyryl-ACP in vitro were purified. These proteins were malonyl-CoA:ACP transacylase (fabD), beta-**ketoacyl -ACP synthase** III (fabH), beta-ketoacyl-ACP reductase (fabG), beta-hydroxydecanoyl-ACP dehydrase (fabA), and enoyl-ACP reductase (fabI). Unlike the other enzymes in the cycle, FabA did not efficiently convert its substrate beta-hydroxybutyryl-ACP to crotonyl-ACP, but rather the equilibrium favored formation of beta-hydroxybutyryl-ACP over crotonyl-ACP by a ratio of 9:1. The amount of butyryl-ACP formed depended on the amount of FabI protein added to the assay. Extracts from fabI(Ts) **mutants** accumulated beta-hydroxybutyryl-ACP, and the addition of FabI protein to the fabI(Ts) extract restored both butyryl-ACP and long-chain acyl-ACP synthesis. FabI was verified to be the only enoyl-ACP reductase required for the synthesis of fatty acids by demonstrating that purified FabI was required for the elongation of both long-chain saturated and unsaturated fatty acids. These results were corroborated by analysis of the intracellular ACP pool composition in fabI(Ts) **mutants** that showed beta-hydroxybutyryl-ACP and crotonyl-ACP accumulated at the nonpermissive temperature in the same ratio found in the fabI(Ts) extracts and in the in vitro reconstruction experiments that lacked FabI. We conclude that FabI is the only enoyl-ACP reductase involved in fatty acid synthesis in E. **coli** and that the activity of this enzyme plays a determinant role in completing cycles of fatty acid biosynthesis.

L5 ANSWER 14 OF 57 MEDLINE
ACCESSION NUMBER: 96042102 MEDLINE
DOCUMENT NUMBER: 96042102 PubMed ID: 7592391
TITLE: Characterization of the polyketide synthase gene (pksL1)

required for aflatoxin biosynthesis in Aspergillus parasiticus.
AUTHOR: Feng G H; Leonard T J
CORPORATE SOURCE: Department of Genetics, University of Wisconsin, Madison 53706, USA.
CONTRACT NUMBER: GM07133 (NIGMS)
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Nov) 177 (21) 6246-54. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L42765; GENBANK-L42766
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951207

AB Aflatoxins are potent toxic and carcinogenic compounds, produced by Aspergillus parasiticus and A. flavus as secondary metabolites. In this research, a polyketide synthase gene (pksL1), the key gene for aflatoxin biosynthesis initiation in A. parasiticus, has been functionally identified and molecularly characterized. PCR-derived DNA probes were used to find the pksL1 gene from subtracted, aflatoxin-related clones. Gene knockout experiments generated four pksL1 disruptants which lost both the ability to produce aflatoxins B1, B2, and G1 and the ability to accumulate norsolorinic acid and all other intermediates of the aflatoxin biosynthetic pathway. A pksL1 DNA probe detected a 6.6-kb poly(A)+ RNA transcript in Northern (RNA) hybridizations. This transcript, associated with aflatoxin production, exhibited a regulated expression that was influenced by growth phase, medium composition, and culture temperature. DNA sequencing of pksL1 revealed an open reading frame for a polypeptide (PKSL1) of 2,109 amino acids. Sequence analysis further recognized four functional domains in PKSL1, acyl carrier protein, beta-**ketoacyl -acyl carrier protein** synthase, acyltransferase, and thioesterase, all of which are usually present in polyketide synthases and fatty acid synthases. On the basis of these results, we propose that pksL1 encodes the polyketide synthase which synthesizes the backbone polyketide and initiates aflatoxin biosynthesis. In addition, the transcript of pksL1 exhibited heterogeneity at the polyadenylation site similar to that of **plant** genes.

L5 ANSWER 15 OF 57 MEDLINE
ACCESSION NUMBER: 95361916 MEDLINE
DOCUMENT NUMBER: 95361916 PubMed ID: 7635191
TITLE: Lipid biosynthetic genes and a ribosomal protein gene are cotranscribed.
AUTHOR: Podkovyrov S; Larson T J
CORPORATE SOURCE: Department of Biochemistry and Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg 24061-0308, USA.
CONTRACT NUMBER: GM47270 (NIGMS)
SOURCE: FEBS LETTERS, (1995 Jul 24) 368 (3) 429-31. Journal code: EUH; 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 19980206
Entered Medline: 19950908

AB By using insertional **mutagenesis** we demonstrated that the rpmF gene encoding ribosomal protein L32, the plsX gene encoding a protein involved in membrane lipid synthesis and several fatty acid biosynthetic genes (fabH, fabD and fabG) are cotranscribed. Organization of these genes into an operon may play a role in the coordinate regulation of the synthesis of ribosomes and the cell membranes.

L5 ANSWER 16 OF 57 MEDLINE
ACCESSION NUMBER: 95332197 MEDLINE
DOCUMENT NUMBER: 95332197 PubMed ID: 7608065
TITLE: Purification of a malonyltransferase from Streptomyces coelicolor A3(2) and analysis of its genetic determinant.
AUTHOR: Revill W P; Bibb M J; Hopwood D A
CORPORATE SOURCE: John Innes Centre, Norwich Research Park, Colney, United Kingdom.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Jul) 177 (14) 3946-52. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-X86475
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950828
Last Updated on STN: 19950828
Entered Medline: 19950815

AB Streptomyces coelicolor A3(2) synthesizes each half molecule of the dimeric polyketide antibiotic actinorhodin (Act) from one acetyl and seven malonyl building units, catalyzed by the Act polyketide synthase (PKS). The synthesis is analogous to fatty acid biosynthesis, and there is evident structural similarity between PKSs of Streptomyces spp. and fatty acid synthases (FASs). Each system should depend on a malonyl coenzyme A:acyl carrier protein malonyltransferase, which charges the FAS or PKS with the malonyl units for carbon chain extension. We have purified the Act acyl carrier protein-dependent malonyltransferase from stationary-phase, Act-producing cultures and have determined the N-terminal amino acid sequence and cloned the structural gene. The deduced amino acid sequence resembles those of known malonyltransferases of FASs and PKSs. The gene lies some 2.8 Mb from the rest of the act cluster, adjacent to an open reading frame whose gene product resembles **ketoacylsynthase** III of Escherichia **coli** FAS. The malonyltransferase was expressed equally as well during vegetative growth (when other components of the act PKS were not expressed) as in the stationary phase, suggesting that the malonyltransferase may be shared between the FAS and PKS of S. coelicolor. Disruption of the operon containing the malonyltransferase gene proved to be impossible, supporting the idea that the malonyltransferase plays an essential role in fatty acid biosynthesis.

L5 ANSWER 17 OF 57 MEDLINE
ACCESSION NUMBER: 95318134 MEDLINE
DOCUMENT NUMBER: 95318134 PubMed ID: 7797547
TITLE: Regulation of malonyl-CoA metabolism by acyl-acyl carrier protein and beta-**ketoacyl-acyl carrier protein** synthases in Escherichia **coli**.
AUTHOR: Heath R J; Rock C O
CORPORATE SOURCE: Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101, USA.
CONTRACT NUMBER: CA 21765 (NCI)
GM34496 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Jun 30) 270 (26) 15531-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950817
Last Updated on STN: 19980206
Entered Medline: 19950801

AB The cessation of phospholipid biosynthesis by the inhibition of the sn-glycerol-3-phosphate acyltransferase using a plsB **mutant** led to an accumulation of long chain acyl-acyl carrier proteins (acyl-ACP) and the concomitant inhibition of de novo fatty acid biosynthesis in Escherichia **coli**. Malonyl-CoA did not accumulate when phospholipid and fatty acid synthesis was blocked. However, the inactivation of beta-ketoacyl-ACP synthases I and II with the antibiotic cerulenin triggered a large increase in the accumulation of malonyl-CoA following the cessation of phospholipid synthesis, illustrating that the beta-ketoacyl-ACP synthases were responsible for the degradation of malonyl-CoA in the presence of long chain acyl-ACP. The acyl-ACP requirement for malonyl-CoA degradation activity was confirmed by shifting enoyl-ACP reductase **mutants** (fabI(Ts)) to the non-permissive temperature, leading to the abrupt cessation of fatty acid synthesis and the accumulation of malonyl-CoA in the absence of cerulenin. Analysis of the ACP pool composition before and after the temperature shift showed that the fabI block did not result in the accumulation of long chain acyl-ACP. These data indicate a feedback regulatory loop that functions to recycle malonyl-CoA to acetyl-CoA following the down-regulation of fatty acid and phospholipid formation and provides a physiological rationale for the acyl-ACP-dependent, malonyl-ACP decarboxylase reaction catalyzed by beta-ketoacyl-ACP synthases I and II.

L5 ANSWER 18 OF 57 MEDLINE
ACCESSION NUMBER: 95252821 MEDLINE
DOCUMENT NUMBER: 95252821 PubMed ID: 7734965
TITLE: Directed tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) gene with the maize transposon activator.
AUTHOR: James D W Jr; Lim E; Keller J; Plooy I; Ralston E; Dooner H K
CORPORATE SOURCE: DNA Plant Technology Corporation, Oakland, California

94608.
SOURCE: PLANT CELL, (1995 Mar) 7 (3) 309-19.
Journal code: BJU; 9208688. ISSN: 1040-4651.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U29142
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950615
Last Updated on STN: 19990129
Entered Medline: 19950607

AB The FATTY ACID ELONGATION1 (FAE1) gene of Arabidopsis is required for the synthesis of very long chain fatty acids in the seed. The product of the FAE1 gene is presumed to be a **condensing enzyme** that extends the chain length of fatty acids from C18 to C20 and C22. We report here the cloning of FAE1 by directed transposon tagging with the maize element Activator (Ac). An unstable fae1 **mutant** was isolated in a line carrying Ac linked to the FAE1 locus on chromosome 4. Cosegregation and reversion analyses established that the new **mutant** was tagged by Ac. A DNA fragment flanking Ac was cloned by inverse polymerase chain reaction and used to isolate FAE1 genomic clones and a cDNA clone from a library made from immature siliques. The predicted amino acid sequence of the FAE1 protein shares homology with those of other condensing enzymes (chalcone synthase, stilbene synthases, and beta-**ketoacyl-acyl carrier protein** synthase III), supporting the notion that FAE1 is the structural gene for a synthase or **condensing enzyme**. FAE1 is expressed in developing seed, but not in leaves, as expected from the effect of the fae1 **mutation** on the fatty acid compositions of those tissues.

L5 ANSWER 19 OF 57 MEDLINE
ACCESSION NUMBER: 95014072 MEDLINE
DOCUMENT NUMBER: 95014072 PubMed ID: 7928998
TITLE: Isolation and sequence analysis of polyketide synthase genes from the daunomycin-producing Streptomyces sp. strain C5.
AUTHOR: Ye J; Dickens M L; Plater R; Li Y; Lawrence J; Strohl W R
CORPORATE SOURCE: Department of Microbiology, Ohio State University, Columbus 43210-1292.
CONTRACT NUMBER: 43345
SOURCE: JOURNAL OF BACTERIOLOGY, (1994 Oct) 176 (20) 6270-80.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L34880
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941110

AB A contiguous region of about 30 kbp of DNA putatively encoding reactions in daunomycin biosynthesis was isolated from Streptomyces sp. strain C5 DNA. The DNA sequence of an 8.1-kbp EcoRI fragment, which hybridized with actI polyketide synthase (PKS) and actIII polyketide reductase (PKR) gene probes, was determined, revealing seven complete open reading frames (ORFs), two in one cluster and five in a divergently transcribed cluster. The former two genes are likely to encode PKR and a bifunctional cyclase/dehydrase. The five latter genes encode: (i) a homolog of TcmH, an oxygenase of the tetracenomycin biosynthesis pathway; (ii) a PKS Orf1 homolog; (iii) a PKS Orf2 homolog (chain length factor); (iv) a product having moderate sequence identity with Escherichia **coli** beta-**ketoacyl acyl carrier protein** synthase III but lacking the conserved active site; and (v) a protein highly similar to several acyltransferases. The DNA within the 8.1-kbp EcoRI fragment restored daunomycin production to two dauA non-daunomycin-producing **mutants** of Streptomyces sp. strain C5 and restored wild-type antibiotic production to Streptomyces coelicolor B40 (act VII; nonfunctional cyclase/dehydrase), and to S. coelicolor B41 (actIII) and Streptomyces galilaeus ATCC 31671, strains defective in PKR activity.

L5 ANSWER 20 OF 57 MEDLINE
ACCESSION NUMBER: 94291939 MEDLINE
DOCUMENT NUMBER: 94291939 PubMed ID: 8020746
TITLE: Identification of the rpmF-plsX-fabH genes of Rhodobacter capsulatus.
AUTHOR: Carty S M; Colbeau A; Vignais P M; Larson T J
CORPORATE SOURCE: Department of Biochemistry and Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg 24061-0308.

SOURCE: FEMS MICROBIOLOGY LETTERS, (1994 May 15) 118 (3) 227-31.
Journal code: FML; 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940815
Last Updated on STN: 19980206
Entered Medline: 19940803

AB The rpmF-plsX-fabH gene cluster of Rhodobacter capsulatus homologous to that of Escherichia **coli** was identified. rpmF encodes ribosomal protein L32, plsX plays an undefined role in membrane lipid synthesis, and fabH encodes beta-**ketoacyl-acyl carrier protein** synthase III. The R. capsulatus plsX gene complemented a defect in an E. **coli** strain with the plsX50 **mutation**. Overproduction of the fabH gene product of R. capsulatus in E. **coli** resulted in dramatically increased beta-**ketoacyl-acyl carrier protein** synthase III activity. These results indicate that plsX and fabH apparently function the same in R. capsulatus as in E. **coli**.

L5 ANSWER 21 OF 57 MEDLINE
ACCESSION NUMBER: 94018649 MEDLINE
DOCUMENT NUMBER: 94018649 PubMed ID: 8412701
TITLE: Identification of a new nuclear gene (CEM1) encoding a protein homologous to a beta-keto-acyl synthase which is essential for mitochondrial respiration in Saccharomyces cerevisiae.
AUTHOR: Harington A; Herbert C J; Tung B; Getz G S; Slonimski P P
CORPORATE SOURCE: Centre de Genetique Moleculaire du CNRS, Laboratoire propre associe a l'Universite Pierre et Marie Curie, Gif-sur-Yvette, France.
CONTRACT NUMBER: HL0-4442 (NHLBI)
SOURCE: MOLECULAR MICROBIOLOGY, (1993 Aug) 9 (3) 545-55.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-UNKNOWN
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19980206
Entered Medline: 19931109

AB We have analysed a new gene, CEM1, from Saccharomyces cerevisiae. Inactivation of this gene leads to a respiratory-deficient phenotype. The deduced protein sequence shows strong similarities with beta-keto-acyl synthases or condensing enzymes. Typically, enzymes of this class are involved in the synthesis of fatty acids or similar molecules. An analysis of the mitochondrial lipids and fatty acids shows no major difference between the wild type and deleted strains, implying that the CEM1 gene product is not involved in the synthesis of the bulk fatty acids. Thus it is possible that the CEM1 protein is involved in the synthesis of a specialized molecule, probably related to a fatty acid, which is essential for mitochondrial respiration.

L5 ANSWER 22 OF 57 MEDLINE
ACCESSION NUMBER: 93285984 MEDLINE
DOCUMENT NUMBER: 93285984 PubMed ID: 8509326
TITLE: Thiolactomycin resistance in Escherichia **coli** is associated with the multidrug resistance efflux pump encoded by emrAB.
AUTHOR: Furukawa H; Tsay J T; Jackowski S; Takamura Y; Rock C O
CORPORATE SOURCE: Department of Agricultural Chemistry, Faculty of Agriculture, Ibaraki University, Japan.
CONTRACT NUMBER: CA 21765 (NCI)
GM 34496 (NIGMS)
SOURCE: JOURNAL OF BACTERIOLOGY, (1993 Jun) 175 (12) 3723-9.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930713

AB Thiolactomycin (TLM) and cerulenin are antibiotics that block Escherichia **coli** growth by inhibiting fatty acid biosynthesis at the beta-**ketoacyl-acyl carrier protein** synthase I step. Both TLM and cerulenin trigger the accumulation of

intracellular malonyl-coenzyme A coincident with growth inhibition, and the overexpression of synthase I protein confers resistance to both antibiotics. Strain CDM5 was derived as a TLM-resistant **mutant** but remained sensitive to cerulenin. TLM neither induced malonyl-coenzyme A accumulation nor blocked fatty acid production in vivo; however, the fatty acid synthase activity in extracts from strain CDM5 was sensitive to TLM inhibition. The TLM resistance gene in strain CDM5 was mapped to 57.5 min of the chromosome and was an allele of the emrB gene. Disruption of the emrB gene converted strain CDM5 to a TLM-sensitive strain, and the overexpression of the emrAB operon conferred TLM resistance to sensitive strains. Thus, activation of the emr efflux pump is the mechanism for TLM resistance in strain CDM5.

L5 ANSWER 23 OF 57 MEDLINE
ACCESSION NUMBER: 93229805 MEDLINE
DOCUMENT NUMBER: 93229805 PubMed ID: 8471795
TITLE: Interaction of Xanthomonas campestris with Arabidopsis thaliana: characterization of a gene from X. c. pv. raphani that confers avirulence to most A. thaliana accessions.
AUTHOR: Parker J E; Barber C E; Fan M J; Daniels M J
CORPORATE SOURCE: Sainsbury Laboratory, John Innes Centre, Norwich Research Park, U.K.
SOURCE: MOLECULAR PLANT-MICROBE INTERACTIONS, (1993 Mar-Apr) 6 (2) 216-24.
Journal code: A9P; 9107902. ISSN: 0894-0282.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M99059
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930604
Last Updated on STN: 19930604
Entered Medline: 19930520

AB Infiltration of leaves of Arabidopsis thaliana accession Columbia with Xanthomonas campestris pathovar campestris leads to bacterial growth and disease symptoms reminiscent of those incited in Brassica plants inoculated under the same conditions. A search among A. thaliana accessions for variation in the reaction phenotype to strains of X. campestris pathovars campestris, aberrans, and raphani showed that there were no clear differential responses between **plant** accessions to the individual bacterial strains tested. X. c. pv. raphani strain 1067 was avirulent to all A. thaliana accessions tested. A gene was cloned from X. c. pv. raphani 1067 which, when transferred into the virulent X. c. pv. campestris strain 8004, strongly reduced symptom development and bacterial growth in A. thaliana Columbia plants but did not affect virulence to Brassica plants. The gene (denoted avrXca) interacted with all A. thaliana accessions tested except one, **Kas**-1, which developed disease symptoms and supported growth of the transconjugant to levels similar to those with X. c. pv. campestris 8004 alone. Sequence analysis of avrXca revealed a probable open reading frame encoding a protein of 66,566 Da that has no homology with other known sequences. A sequence motif conserved among hrp genes was identified in the 5' noncoding region of avrXca, and features characteristic of a signal peptide were found in the N-terminal portion of the presumed AvrXca protein. DNA from different phytopathogenic bacteria contained sequences hybridizing with avrXca in related X. campestris pathovars but not in Erwinia or Pseudomonas strains.

L5 ANSWER 24 OF 57 MEDLINE
ACCESSION NUMBER: 92406871 MEDLINE
DOCUMENT NUMBER: 92406871 PubMed ID: 1527048
TITLE: Nucleotide sequence and deduced functions of a set of cotranscribed genes of Streptomyces coelicolor A3(2) including the polyketide synthase for the antibiotic actinorhodin.
AUTHOR: Fernandez-Moreno M A; Martinez E; Boto L; Hopwood D A; Malpartida F
CORPORATE SOURCE: Centro Nacional de Biotecnologia, Madrid, Spain.
CONTRACT NUMBER: GM 39784 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Sep 25) 267 (27) 19278-90.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D10347; GENBANK-D10348; GENBANK-D11148; GENBANK-D11149; GENBANK-D11150; GENBANK-D11151; GENBANK-S76077; GENBANK-S76125; GENBANK-S76126; GENBANK-X63449; GENBANK-X63499
ENTRY MONTH: 199210
ENTRY DATE: Entered STN: 19921106

Last Updated on STN: 19921106
Entered Medline: 19921022

AB A 5.3-kb region of the Streptomyces coelicolor actinorhodin gene cluster, including the genes for polyketide biosynthesis, was sequenced. Six identified open reading frames (ORF1-6) were related to genetically characterized **mutations** of classes actI, VII, IV, and VB by complementation analysis. ORF1-6 run divergently from the adjacent actIII gene, which encodes the polyketide synthase (PKS) ketoreductase, and appear to form an operon. The deduced gene products of ORF1-3 are similar to fatty acid synthases (FAS) of different organisms and PKS genes from other polyketide producers. The predicted ORF5 gene product is similar to type II beta-lactamases of Bacillus cereus and Bacteroides fragilis. The ORF6 product does not resemble other known proteins. Combining the genetical, biochemical, and similarity data, the potential activities of the products of the six genes can be postulated as: 1) **condensing enzyme**/acyl transferase (ORF1 + ORF2); 2) acyl carrier protein (ORF3); 3) putative cyclase/dehydrase (ORF4); 4) dehydrase (ORF5); and 5) "dimerase" (ORF6). The data show that the actinorhodin PKS consists of discrete monofunctional components, like that of the Escherichia **coli** (Type II) FAS, rather than the multifunctional polypeptides for the macrolide PKSs and vertebrate FASs (Type I).

L5 ANSWER 25 OF 57 MEDLINE
ACCESSION NUMBER: 92234941 MEDLINE
DOCUMENT NUMBER: 92234941 PubMed ID: 1314802
TITLE: Cloning, nucleotide sequence, and expression of the Escherichia **coli** fabD gene, encoding malonyl coenzyme A-acyl carrier protein transacylase.
AUTHOR: Verwoert I I; Verbree E C; van der Linden K H; Nijkamp H J; Stuitje A R
CORPORATE SOURCE: Department of Genetics, Vrije Universiteit, Amsterdam, The Netherlands.
SOURCE: JOURNAL OF BACTERIOLOGY, (1992 May) 174 (9) 2851-7.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M79367; GENBANK-M79368; GENBANK-M79369; GENBANK-M79370; GENBANK-M79371; GENBANK-M79372; GENBANK-M81469; GENBANK-M87040; GENBANK-M89479; GENBANK-M89480
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920612
Last Updated on STN: 19920612
Entered Medline: 19920522

AB The Escherichia **coli** fabD gene encoding malonyl coenzyme A-acyl carrier protein transacylase (MCT) was cloned by complementation of a thermosensitive E. **coli** fabD **mutant** (fabD89). Expression of the fabD gene in an appropriate E. **coli** expression vector resulted in an accumulation of the MCT protein of up to 10% of total soluble protein, which was accompanied by an approximately 1,000-fold increase in the MCT activity. DNA sequence analysis and expression studies revealed that the fabD gene is part of an operon consisting of at least three genes involved in fatty acid biosynthesis. Comparison with available DNA and protein data bases suggest that a 3-**ketoacyl-acyl carrier protein** synthase and a **ketoacyl-acyl carrier protein** reductase gene are located immediately upstream and downstream, respectively, of fabD within this fab operon. Western immunoblot analysis with antiserum raised against wild-type E. **coli** MCT showed that the fabD89 allele encodes a polypeptide with an apparent molecular weight of 27,000 in addition to the normal MCT protein of 32,000. The nature of the temperature-sensitive fabD89 gene product is discussed.

L5 ANSWER 26 OF 57 MEDLINE
ACCESSION NUMBER: 92202232 MEDLINE
DOCUMENT NUMBER: 92202232 PubMed ID: 1551888
TITLE: Isolation and characterization of the beta-**ketoacyl -acyl carrier protein** synthase III gene (fabH) from Escherichia **coli** K-12.
AUTHOR: Tsay J T; Oh W; Larson T J; Jackowski S; Rock C O
CORPORATE SOURCE: Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38105.
CONTRACT NUMBER: CA 21765 (NCI)
GM 34496 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Apr 5) 267 (10) 6807-14.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L01675; GENBANK-L01676; GENBANK-L01677; GENBANK-M77744; GENBANK-M81836; GENBANK-M83679; GENBANK-M83680; GENBANK-M83681; GENBANK-M83724; GENBANK-M96793
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 19920509
Last Updated on STN: 19980206
Entered Medline: 19920428

AB beta-**Ketoacyl-acyl carrier protein** (ACP) synthase III catalyzes the condensation of acetyl-CoA with malonyl-ACP in dissociated (Type II) fatty acid synthase systems. A synthase III **mutant** was used to localize the structural gene to the 24.5-min region of the Escherichia **coli** chromosome, and the defective synthase III allele was designated fabH1. The fabH gene was identified on a 1.3-kilobase NruI-HindIII chromosomal DNA fragment (plasmid pWO114) that complemented the enzymatic defect in fabH1 strains. The NruI-HindIII fragment was sequenced and contained a single open reading frame predicted to encode a 33,517-dalton protein with an isoelectric point of 4.85. The fabH sequence contained an Ala-Cys-Ala tripeptide characteristic of **condensing enzyme** active sites. A T7 expression system showed that the NruI-HindIII fragment directed the synthesis of a single 34,800-dalton protein. This protein was purified and the order of the amino-terminal 30 residues of the protein corresponded exactly to the amino acid structure predicted from the DNA sequence. The purified protein possessed both acetoacetyl-ACP synthase and acetyl-CoA:ACP transacylase activities, and cells harboring plasmid pWO114 overproduced the two activities, supporting the conclusion that a single protein carries out both reactions. Overproduction of synthase III resulted in a significant increase in shorter-chain fatty acids in the membrane phospholipids. These catalytic properties are consistent with the proposed role of synthase III in the initiation of fatty acid synthesis.

L5 ANSWER 27 OF 57 MEDLINE
ACCESSION NUMBER: 92105019 MEDLINE
DOCUMENT NUMBER: 92105019 PubMed ID: 1729241
TITLE: Overproduction of beta-**ketoacyl-acyl carrier protein** synthase I imparts thiolactomycin resistance to Escherichia **coli** K-12.
AUTHOR: Tsay J T; Rock C O; Jackowski S
CORPORATE SOURCE: Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.
CONTRACT NUMBER: CA 21765 (NCI)
GM 34496 (NIGMS)
SOURCE: JOURNAL OF BACTERIOLOGY, (1992 Jan) 174 (2) 508-13.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920302
Last Updated on STN: 19920302
Entered Medline: 19920212

AB Thiolactomycin [(4S)(2E,5E)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide] (TLM) is a unique antibiotic structure that inhibits dissociated type II fatty acid synthase systems but not the multifunctional type I fatty acid synthases found in mammals. We screened an Escherichia **coli** genomic library for recombinant plasmids that impart TLM resistance to a TLM-sensitive strain of E. **coli** K-12. Nine independent plasmids were isolated, and all possessed a functional beta-**ketoacyl-acyl carrier protein** synthase I gene (fabB) based on their restriction enzyme maps and complementation of the temperature-sensitive growth of a fabB15(Ts) **mutant**. A plasmid (pJTB3) was constructed that contained only the fabB open reading frame. This plasmid conferred TLM resistance, complemented the fabB(Ts) **mutation**, and directed the overproduction of synthase I activity. TLM selectively inhibited unsaturated fatty acid synthesis in vivo; however, synthase I was not the only TLM target, since supplementation with oleate to circumvent the cellular requirement for an active synthase I did not confer TLM resistance. Overproduction of the FabB protein resulted in TLM-resistant fatty acid biosynthesis in vivo and in vitro. These data show that beta-**ketoacyl-acyl carrier protein** synthase I is a major target for TLM and that increased expression of this **condensing enzyme** is one mechanism for acquiring TLM resistance. However, extracts from a TLM-resistant **mutant** (strain CDM5) contained normal levels of TLM-sensitive synthase I activity, illustrating that there are other mechanisms of TLM resistance.

L5 ANSWER 28 OF 57 MEDLINE
ACCESSION NUMBER: 89351281 MEDLINE
DOCUMENT NUMBER: 89351281 PubMed ID: 3076377
TITLE: Role of Escherichia **coli** beta-**ketoacyl-ACP synthase** I in unsaturated fatty acid synthesis.
AUTHOR: Siggaard-Andersen M
CORPORATE SOURCE: Department of Physiology, Carlsberg Laboratory, Copenhagen Valby.
SOURCE: CARLSBERG RESEARCH COMMUNICATIONS, (1988) 53 (6) 371-9.
Journal code: ADT; 7703861. ISSN: 0105-1938.
PUB. COUNTRY: Denmark
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198910
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19980206
Entered Medline: 19891011
AB Two activities were found in E. **coli** extracts which could complement unsaturated fatty acid synthesis of a cerulenin treated E. **coli** fatty acid synthetase. One of these is beta-**ketoacyl-ACP synthase** I, but it is not known whether the other activity represents the previously characterized beta-**ketoacyl-ACP synthase** II. A **mutant** strain exhibiting a temperature sensitive unsaturated fatty acid synthetic activity apparently lacked an active beta-**ketoacyl-ACP synthase** I.

L5 ANSWER 29 OF 57 MEDLINE
ACCESSION NUMBER: 89214213 MEDLINE
DOCUMENT NUMBER: 89214213 PubMed ID: 2651445
TITLE: Acetoacetyl-acyl carrier protein synthase. A target for the antibiotic thiolactomycin.
AUTHOR: Jackowski S; Murphy C M; Cronan J E Jr; Rock C O
CORPORATE SOURCE: Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.
CONTRACT NUMBER: CA21765 (NCI)
GM 26156 (NIGMS)
GM 34496 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 May 5) 264 (13) 7624-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198906
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19980206
Entered Medline: 19890602
AB The biochemical basis for the inhibition of fatty acid biosynthesis in Escherichia **coli** by the antibiotic thiolactomycin was investigated. A biochemical assay was developed to measure acetoacetyl-acyl carrier protein (ACP) synthase activity, a recently discovered third **condensing enzyme** from E. **coli** (Jackowski, S., and Rock, C.O. (1987) J. Biol. Chem. 262, 7927-7931). In contrast to the other two condensing enzymes in E. **coli**, acetoacetyl-ACP synthase (synthase III) condensed malonyl-ACP with acetyl-CoA, rather than with acetyl-ACP. The concentration dependence of thiolactomycin inhibition of fatty acid biosynthesis in vivo was the same as the inhibition of acetoacetyl-ACP synthase activity in vitro indicating that the two phenomena were related. A thiolactomycin-resistant **mutant** (strain CDM5) was isolated. The specific activity of acetoacetyl-ACP synthase in extracts from this **mutant** was 10-fold lower than in extracts from its thiolactomycin-sensitive parent resulting in a marked defect in the ability of strain CDM5 to incorporate acetyl-CoA into fatty acids in vitro. The residual acetoacetyl-ACP synthase activity in the resistant strain was refractory to thiolactomycin inhibition. In addition, acetyl-CoA:ACP transacylase activity in strain CDM5 was resistant to inactivation by thiolactomycin suggesting that the acetoacetyl-ACP synthase also catalyzes this transacylation reaction. These data point to acetoacetyl-ACP synthase as a target for thiolactomycin inhibition of bacterial fatty acid biosynthesis.

L5 ANSWER 30 OF 57 MEDLINE
ACCESSION NUMBER: 87222428 MEDLINE
DOCUMENT NUMBER: 87222428 PubMed ID: 3294837
TITLE: Acetoacetyl-acyl carrier protein synthase, a potential regulator of fatty acid biosynthesis in bacteria.

AUTHOR: Jackowski S; Rock C O
CONTRACT NUMBER: CA 21765 (NCI)
GM 34496 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1987 Jun 5) 262 (16) 7927-31.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198707
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19870702

AB The first condensation reaction in the fatty acid biosynthetic pathway in Escherichia **coli** was rate-limiting as judged by analysis of the relative pool sizes of acyl carrier protein (ACP) thioester intermediates in vivo. Comparable concentrations of acetyl-ACP, malonyl-ACP, and nonesterified ACP were present during logarithmic growth, whereas long-chain acyl-ACP comprised a minor fraction of the total ACP pool. The antibiotic cerulenin was used to irreversibly inhibit both beta-ketoacyl-ACP synthases I and II. However, acyl-ACP formation in vivo was not blocked by this antibiotic, and short-chain (4-8-carbon) acyl-ACPs increased to 60% of the total ACP pool in cerulenin-treated cells. These data suggested that existence of a cerulenin-resistant **condensing enzyme** that was capable of catalyzing the initial steps in chain elongation. A unique enzymatic activity, acetoacetyl-ACP synthase, that specifically catalyzed the condensation of malonyl-ACP and acetyl-ACP was detected in E. **coli** cell extracts. Acetoacetyl-ACP synthase activity was not inhibited by cerulenin and was present in extracts prepared from a double **mutant** harboring genetic lesions in beta-ketoacyl-ACP synthases I and II (fabB20 fabF3). These data point to the condensation of malonyl-ACP and acetyl-ACP as the rate-controlling reaction in fatty acid biosynthesis and implicate acetoacetyl-ACP synthase as the pacemaker of fatty acid production in organisms and organelles that possess dissociated (Type II) fatty acid synthase systems.

L5 ANSWER 31 OF 57 MEDLINE
ACCESSION NUMBER: 87165751 MEDLINE
DOCUMENT NUMBER: 87165751 PubMed ID: 3549687
TITLE: Altered molecular form of acyl carrier protein associated with beta-**ketoacyl-acyl carrier protein** synthase II (fabF) **mutants**.
AUTHOR: Jackowski S; Rock C O
CONTRACT NUMBER: CA 21765 (NCI)
GN 34496
SOURCE: JOURNAL OF BACTERIOLOGY, (1987 Apr) 169 (4) 1469-73.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198705
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19980206
Entered Medline: 19870505

AB Acyl carrier protein (ACP) is a required cofactor for fatty acid synthesis in Escherichia **coli**. **Mutants** lacking beta-**ketoacyl-ACP synthase** II activity (fabF1 or fabF3) possessed a different molecular species of ACP (F-ACP) that was separated from the normal form of the protein by conformationally sensitive gel electrophoresis. Synthase I **mutants** contained the normal protein. Complementation of fabF1 **mutants** with an F' factor harboring the wild-type synthase II allele resulted in the appearance of normal ACP, whereas complementation with an F' possessing the fabF2 allele (a **mutation** that produces a synthase II enzyme with altered catalytic activity) resulted in the production of both forms of ACP. The structural difference between F-ACP and ACP persisted after the removal of the 4'-phosphopantetheine prosthetic group, and both forms of the protein had identical properties in an in vitro fatty acid synthase assay. Both ACP and F-ACP were purified to homogeneity, and their primary amino acid sequences were determined. The two ACP species were identical but differed from the sequence reported for E. **coli** E-15 ACP in that an Asn instead of an Asp was at position 24 and an Ile instead of a Val was at position 43. Therefore, F-ACP appears to be a modification of ACP that is detected when beta-**ketoacyl-ACP synthase** II activity is impaired.

L5 ANSWER 32 OF 57 MEDLINE
ACCESSION NUMBER: 86131753 MEDLINE
DOCUMENT NUMBER: 86131753 PubMed ID: 3511968
TITLE: Effects of the temperature range and the lack of beta-

ketoacyl acyl-carrier protein synthase II on fatty acid synthesis in Escherichia **coli** K12 after shifts in temperature.
AUTHOR: Oh-hashi Y; Okuyama H
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1986 Mar 21) 876 (1) 146-53.
Journal code: A0W; 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198604
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19980206
Entered Medline: 19860424

AB Escherichia **coli** K12 cells grown at higher temperatures and then subjected to lower temperatures produce fatty acids with higher unsaturated/saturated ratios than cells completely adapted to the lower temperatures (Okuyama et al. (1982) J. Biol. Chem. 257, 4812-4817). This hyper-response was not an artefact of chloramphenicol treatment and was observed when the shift-down was more than 20 degrees C in the cells grown at either 40 degrees C or 35 degrees C. In contrast, cells grown at either 25 degrees C or 30 degrees C showed no appreciable hyper-response in terms of unsaturated/saturated ratio on temperature shifts to as low as 10 degrees C. By combining shift-down and shift-up experiments, we could show the presence of different types of temperature dependency in the fatty acid-synthesizing systems of cells grown at various temperatures. Contrary to wild-type cells which synthesized mainly cis-vaccenate on down-shift to 10 degrees C, a **mutant** strain lacking beta-**ketoacyl acyl-carrier protein** synthase II synthesized more palmitoleate (16:1) and less palmitate at 10 degrees C than at 40 degrees C. The average chain lengths of saturated and unsaturated fatty acids also changed, but differently, between the **mutant** and wild-type cells on shifts of temperature. Thus, the **mutant** strain has a temperature-dependent fatty acid-synthesizing system qualitatively different from that seen in a wild-type strain.

L5 ANSWER 33 OF 57 MEDLINE
ACCESSION NUMBER: 84104816 MEDLINE
DOCUMENT NUMBER: 84104816 PubMed ID: 6362720
TITLE: Beta-hydroxydecanoyl thio ester dehydrase does not catalyze a rate-limiting step in Escherichia **coli** unsaturated fatty acid synthesis.
AUTHOR: Clark D P; DeMendoza D; Polacco M L; Cronan J E Jr
CONTRACT NUMBER: AI 15650 (NIAID)
AM05390 (NIADDK)
SOURCE: BIOCHEMISTRY, (1983 Dec 6) 22 (25) 5897-902.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198403
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19840323

AB The intracellular level of beta-hydroxydecanoyl thio ester dehydrase, the product of the fabA gene of Escherichia **coli**, was increased by isolation of a putative promotor **mutant** (termed fabAup) or by molecular cloning of the wild-type fabA gene into plasmid pBR322. The fabAup and plasmid-carrying strains overproduced dehydrase by about 15- and 10-fold, respectively. The phospholipids of all strains that overproduced the dehydrase contained significantly higher levels of saturated fatty acids than isogenic strains producing a normal level of dehydrase. No increased levels of unsaturated fatty acids were observed. This result indicates that, although the dehydrase is required for unsaturated fatty acid synthesis, the level of dehydrase activity in wild-type cells does not limit the rate of unsaturated fatty acid synthesis. The introduction of a plasmid carrying the structural gene for beta-**ketoacyl acyl carrier protein** synthase I into a fabAup strain overcame the effect of dehydrase overproduction on fatty acid composition.

L5 ANSWER 34 OF 57 MEDLINE
ACCESSION NUMBER: 83160777 MEDLINE
DOCUMENT NUMBER: 83160777 PubMed ID: 6339472
TITLE: Genetic and biochemical analyses of Escherichia **coli mutants** altered in the temperature-dependent regulation of membrane lipid composition.
AUTHOR: Ulrich A K; de Mendoza D; Garwin J L; Cronan J E Jr
CONTRACT NUMBER: AI15650 (NIAID)

SOURCE: JOURNAL OF BACTERIOLOGY, (1983 Apr) 154 (1) 221-30.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198305
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 19980206
Entered Medline: 19830505

L5 ANSWER 35 OF 57 MEDLINE
ACCESSION NUMBER: 81069887 MEDLINE
DOCUMENT NUMBER: 81069887 PubMed ID: 7002930
TITLE: Structural, enzymatic, and genetic studies of beta-**ketoacyl-acyl carrier protein** synthases I and II of Escherichia **coli**.
AUTHOR: Garwin J L; Klages A L; Cronan J E Jr
CONTRACT NUMBER: AI 15650 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1980 Dec 25) 255 (24) 11949-56.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198102
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19980206
Entered Medline: 19810219

AB Beta-**Ketoacyl-acyl carrier protein** synthases I and II of Escherichia **coli** were purified and characterized. Synthase I was shown to have a molecular weight of 80,000 +/- 5,000 and to be composed of two similarly sized subunits. Synthase II had a molecular weight of 85,000 +/- 5,000 and also was apparently homodimeric. Gel electrophoresis of partial proteolytic digests demonstrated that synthases I and II share few if any common peptides. Synthases I and II also were shown to be unrelated by immunological criteria. An improved assay for beta-**ketoacyl-acyl carrier protein** synthase activity gave kinetic parameters for synthases I and II at both 27 degrees C and 37 degrees C using five long chain acyl-acyl carrier protein substrates. The properties of synthase II are consistent with the proposed role of this enzyme in the modulation of fatty acid synthesis by temperature. fabF **mutants** of E. **coli** lack synthase II. The fabF locus was mapped at min 24.5 of the E. **coli** genetic map and the clockwise map order was found to be pyrC, fabD, fabF, purB.

L5 ANSWER 36 OF 57 MEDLINE
ACCESSION NUMBER: 80262494 MEDLINE
DOCUMENT NUMBER: 80262494 PubMed ID: 6447436
TITLE: Reversible effects of ethanol on E. **coli**.
AUTHOR: Ingram L O; Dickens B F; Buttke T M
SOURCE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1980) 126 299-337.
Journal code: 2LU; 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198010
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19980206
Entered Medline: 19801027

AB Chronic exposure of E. **coli** to ethanol during growth resulted in major changes in lipid composition. These ethanol-induced changes, a decrease in the proportion of saturated fatty acids, are similar to those which occur following a shift to lower temperature. Products of ethanol metabolism such as acetaldehyde and acetate caused the opposite changes in fatty acid composition. In vivo studies using **mutants** blocked in lipid synthesis indicated that saturated fatty acid synthesis was the primary target leading to changes in bulk lipid fatty acid composition. This was confirmed in vitro and **condensing enzyme** II was identified as the probable site of ethanol inhibition. The acute affects of ethanol on the function of two membrane-bound enzymes, Mg++ATPase and lac permease were also examined. In both cases, cells grown in the presence of ethanol. In time-course studies, permease function was restored concurrently with changes in lipid composition. **Mutants** were isolated which were able to grow in the presence of high levels of ethanol. These **mutants** displayed exaggerated changes in lipid composition providing evidence that alcohol-resistance and fatty acid

changes are related.

L5 ANSWER 37 OF 57 MEDLINE
ACCESSION NUMBER: 80159932 MEDLINE
DOCUMENT NUMBER: 80159932 PubMed ID: 6988423
TITLE: Beta-**ketoacyl-acyl carrier protein** synthase II of Escherichia **coli**. Evidence for function in the thermal regulation of fatty acid synthesis.
AUTHOR: Garwin J L; Klages A L; Cronan J E Jr
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1980 Apr 25) 255 (8) 3263-5.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198006
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19980206
Entered Medline: 19800625
AB Cvc- **mutants** of Escherichia **coli** are deficient in the synthesis of cis-vaccenic acid and in the temperature control of fatty acid synthesis. In this communication, it is demonstrated that these **mutants** lack beta-**ketoacyl-acyl carrier protein** synthase II. The deficiencies in cis-vaccenate synthesis and synthase II are shown to be due to a lesion in the same gene, fabF. Lesions in the fabF gene are found to affect growth only when the strain also carries a lesion in the fabB gene, the structural gene for beta-**ketoacyl-acyl carrier protein** synthase I.

L5 ANSWER 38 OF 57 MEDLINE
ACCESSION NUMBER: 79082790 MEDLINE
DOCUMENT NUMBER: 79082790 PubMed ID: 365224
TITLE: Inhibition of unsaturated fatty acid synthesis in escherichia **coli** by the antibiotic cerulenin.
AUTHOR: Buttke T M; Ingram L O
SOURCE: BIOCHEMISTRY, (1978 Nov 28) 17 (24) 5282-6.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197903
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19980206
Entered Medline: 19790324
AB Low concentrations of cerulenin inhibit the growth of Escherichia **coli** by selectively blocking unsaturated fatty acid synthesis. This inhibition was relieved by unsaturated fatty acid supplements alone but not by saturated fatty acid supplements. The utilization of exogenous unsaturated fatty acids to sustain growth in the presence of cerulenin was confirmed by the analysis of bulk lipid composition. The effects of cerulenin on fatty acid synthesis were examined in vivo by pulse labeling with [14C]acetate and in vitro using [14C]malonyl-coenzyme A. In both cases, unsaturated fatty acid synthesis was inhibited by low concentrations of cerulenin with a stimulation of saturated fatty acid synthesis. Using **mutant** strains deficient in fatty acid synthesis, the effects of cerulenin on beta-**ketoacyl-**[**acyl-carrier-protein**] synthetases I and II were examined. Our results indicate that beta-**ketoacyl-**[**acyl-carrier-protein**] synthetase I is more sensitive to inhibition by cerulenin than beta-**ketoacyl-**[**acyl-carrier-protein**] synthetase II.

L5 ANSWER 39 OF 57 MEDLINE
ACCESSION NUMBER: 75189456 MEDLINE
DOCUMENT NUMBER: 75189456 PubMed ID: 237914
TITLE: Multiple forms of beta-**ketoacyl-acyl carrier protein** synthetase in Escherichia **coli**.
AUTHOR: D'Agnolo G; Rosenfeld I S; Vagelos P R
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1975 Jul 25) 250 (14) 5289-94.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197510
ENTRY DATE: Entered STN: 19900310

Last Updated on STN: 19980206
Entered Medline: 19751010

AB Two forms of beta-**ketoacyl-acyl carrier protein** (ACP) synthetase (designated I and II) have been identified in extracts of Escherichia **coli**. Synthetase I corresponds to the **condensing enzyme** that was studied earlier (GREENSPAN, M.D., ALBERTS, A.W., and VAGELOS, P.R. (1969) J. Biol. Chem. 244, 6477-6485); synthetase II represents a new form of the enzyme. Synthetase II was isolated as a homogeneous protein. It differs from synthetase I in having a higher molecular weight (76,999 versus 66,000), a lower pH optimum (5.5 to 6.1 versus 7.2), and a greater resistance to denaturation by heat. Synthetase II is similar to synthetase I in that both are inactivated by iodoacetamide, and prior incubation of the enzymes with fatty acyl thioesters prevents the inhibitory effect of iodoacetamide. Both also react with a fatty acyl thioester to form an acyl-enzyme intermediate, and the latter reacts with malonyl-ACP to form a beta-ketoacyl thioester. Specificity studies indicated that synthetase II, like synthetase I, has similar affinities with saturated and cis unsaturated fatty acyl thioesters of ACP that are intermediates in the synthesis of saturated and unsaturated fatty acids, respectively. The two synthetases differ only with respect to reactivity with palmitoleyl thioesters: synthetase II has a lower Km and higher Vmax than synthetase I with palmitoleyl-ACP. This finding suggests that synthetase II functions specifically in the elongation of palmitoleyl-ACP to form cis-vaccenyl-ACP. An investigation of synthetases I and II in two classes of unsaturated fatty acid auxotrophs revealed that synthetase I is absent in one class, fabB. Addition of wild type synthetase I to fabB fatty acid synthetase, which synthesizes only saturated fatty acids, permitted this fatty acid synthetase to synthesize unsaturated fatty acids. These experiments indicate that synthetase I plays a critical role in the synthesis of unsaturated fatty acids.

L5 ANSWER 40 OF 57 AGRICOLA
ACCESSION NUMBER: 1999:72183 AGRICOLA
DOCUMENT NUMBER: IND22008226
TITLE: Enzymatic characterisation of high-palmitic acid sunflower (Helianthus annuus L.) **mutants**.
AUTHOR(S): Martinez-Force, E.; Alvarez-Ortega, R.; Garces, R.
CORPORATE SOURCE: Consejo Superior de Investigaciones Cinetificas, Sevilla, Spain.
AVAILABILITY: DNAL (450 P693)
SOURCE: Planta, Feb 1999. Vol. 207, No. 4. p. 533-538
Publisher: Berlin ; New York : Springer-Verlag, 1925-
CODEN: PLANAB; ISSN: 0032-0935
NOTE: Includes references
PUB. COUNTRY: Germany
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB Two high-palmitic acid sunflower (Helianthus annuus L.) **mutants**, CAS-5 and CAS-12, have been biochemically characterised. The enzymatic activities found to be responsible for the **mutant** characteristics are beta-keto-acyl-acyl carrier protein synthetase II (KASII; EC **2.3.1.41**) and acyl-acyl carrier protein thioesterase (EC 3.1.2.14). Our data suggest that the high-palmitic acid phenotype observed in both **mutant** lines is due to the combined effect of a lower KASII activity and a higher thioesterase activity with respect to palmitoyl-acyl carrier protein (16:0-ACP). The level of the latter enzyme appeared to be insufficient to hydrolyse the produced 16:0-ACP completely. As a consequence of this, three new fatty acids appear: palmitoleic acid (16:1 delta9), asclepic acid (18:1 delta11), and palmitolinoleic acid (16:2 delta9 delta12). These fatty acids should be synthesised from palmitoyl-ACP or a derivative by the action of the stearoyl-ACP desaturase, fatty acid synthetase II and oleoyl-phosphatidylcholine desaturase, respectively.

L5 ANSWER 41 OF 57 AGRICOLA
ACCESSION NUMBER: 97:41722 AGRICOLA
DOCUMENT NUMBER: IND20571232
TITLE: **Mutation** or increased copy number of nodE has no effect on the spectrum of chitolipooligosaccharide nod factors made by Rhizobium leguminosarum bv. trifolii.
AUTHOR(S): Philip-Hollingsworth, S.; Orgambide, G.G.; Bradford, J.J.; Smith, D.K.; Hollingsworth, R.I.; Dazzo, F.B.
CORPORATE SOURCE: Michigan State University, East Lansing, MI.
AVAILABILITY: DNAL (381 J824)
SOURCE: The Journal of biological chemistry, Sept 8, 1995. Vol. 270, No. 36. p. 20968-20977
Publisher: Bethesda, Md. : American Society for Biochemistry and Molecular Biology.
CODEN: JBCHA3; ISSN: 0021-9258

NOTE: Includes references
PUB. COUNTRY: Maryland; United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB The bacterial gene nodE is the key determinant of host specificity in the Rhizobium leguminosarum-legume symbiosis and has been proposed to determine unique polyunsaturated fatty acyl moieties in chitolipooligosaccharides (CLOS) made by the bacterial symbiont. We evaluated node function by examining CLOS structures made by wild-type R. leguminosarum bv. trifolii ANU843, an isogenic nodE::Tn5 **mutant**, and a recombinant strain containing multiple copies of the pSym nod region of ANU843. 1H-NMR, electrospray ionization mass spectrometry, fast atom bombardment mass spectrometry, flame ionization detection-gas chromatography, gas chromatography/mass spectrometry, and high performance liquid chromatography/UV photodiode array analyses revealed that these bacterial strains made the same spectrum of CLOS species. We also found that ions in the mass spectra which were originally assigned to node-dependent CLOS species containing unique polyunsaturated fatty acids (Spaink, H. P., Bloemberg, G. V., van Brussel, A. A. N., Lugtenberg, B. J. J., van der Drift, K. M. G. M., Haverkamp, J., and Thomas-Oates, J. E. (1995) Mol. **Plant**-Microbe Interact. 8, 155-164) were actually due to sodium adducts of the major nodE-independent CLOS species. No evidence for node-dependent CLOSs was found for these strains. These results indicate a need to revise the current model to explain how node determines host range in the R. leguminosarum-legume symbiosis.

L5 ANSWER 42 OF 57 AGRICOLA
ACCESSION NUMBER: 95:13037 AGRICOLA
DOCUMENT NUMBER: IND20444962
TITLE: A **mutant** of Arabidopsis deficient in the elongation of palmitic acid.
AUTHOR(S): Wu, J.; James, D.W. Jr; Dooner, H.K.; Browse, J.
CORPORATE SOURCE: Virginia Polytechnic Institute and State University, Blacksburg, VA
AVAILABILITY: DNAL (450 P692)
SOURCE: Plant physiology, Sept 1994. Vol. 106, No. 1. p. 143-150
Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-
CODEN: PLPHAY; ISSN: 0032-0889
NOTE: Includes references
PUB. COUNTRY: Maryland; United States
DOCUMENT TYPE: Article; Conference
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB The overall fatty acid composition of leaf lipids in a **mutant** of Arabidopsis thaliana was characterized by an increased level of 16:0 and a concomitant decrease of 18-carbon fatty acids as a consequence of a single recessive nuclear **mutation** at the fab1 locus. Quantitative analysis of the fatty acid composition of individual lipids established that lipids synthesized by both the **prokaryotic** and eukaryotic pathways were affected by the **mutation**. Direct enzyme assays demonstrated that the **mutant** plants were deficient in the activity of 3-**ketoacyl-acyl carrier protein** synthase II; therefore, it is inferred that fab1 may encode this enzyme. Labeling experiments with [14C]acetate and lipase positional analysis indicated that the **mutation** results in a small shift in the partitioning of lipid synthesis between the **prokaryotic** and eukaryotic pathways. Synthesis of chloroplast lipids by the **prokaryotic** pathway was increased with a corresponding reduction in the eukaryotic pathway.

L5 ANSWER 43 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:426541 CAPLUS
DOCUMENT NUMBER: 129:171400
TITLE: Transcriptional analysis of essential genes of the Escherichia **coli** fatty acid biosynthesis gene cluster by functional replacement with the analogous Salmonella typhimurium gene cluster
AUTHOR(S): Zhang, Yan; Cronan, John E., Jr.
CORPORATE SOURCE: Department of Microbiology, University of Illinois, Urbana, IL, 61801, USA
SOURCE: J. Bacteriol. (1998), 180(13), 3295-3303
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The genes encoding several key fatty acid biosynthetic enzymes (called the fab cluster) are clustered in the order plsX-fabH-fabD-fabG-acpP-fabF at min 24 of the Escherichia **coli** chromosome. A difficulty in anal. of the fab cluster by the polar allele duplication approach (Y.

Zhang and J. E. Cronan, Jr., J. Bacteriol. 178:3614-3620, 1996) is that several of these genes are essential for the growth of E. **coli**. We overcame this complication by use of the fab gene cluster of Salmonella typhimurium, a close relative of E. **coli**, to provide functions necessary for growth. The S. typhimurium fab cluster was isolated by complementation of an E. **coli** fabD **mutant** and was found to encode proteins with >94% homol. to those of E. **coli**. However, the S. typhimurium sequences cannot recombine with the E. **coli** sequences required to direct polar allele duplication via homologous recombination. Using this approach, we found that although approx. 60% of the plsX transcripts initiate at promoters located far upstream and include the upstream rpmF ribosomal protein gene, a promoter located upstream of the plsX coding sequence (probably within the upstream gene, rpmF) is sufficient for normal growth. We have also found that the fabG gene is obligatorily cotranscribed with upstream genes. Insertion of a transcription terminator cassette (.OMEGA.-Cm cassette) between the fabD and fabG genes of the E. **coli** chromosome abolished fabG transcription and blocked cell growth, thus providing the first indication that fabG is an essential gene. Insertion of the .OMEGA.-Cm cassette between fabH and fabD caused greatly decreased transcription of the fabD and fabG genes and slower cellular growth, indicating that fabD has only a weak promoter(s).

L5 ANSWER 44 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:707008 CAPLUS
DOCUMENT NUMBER: 123:138894
TITLE: Genetic and biochemical studies of medium chain fatty acid synthesis in Cuphea
AUTHOR(S): Slabaugh, Mary; Leonard, Jeff; Huestis, Gordon; Crane, Jim; Knapp, Steve
CORPORATE SOURCE: Department Crop and Soil Science, Oregon State University, Corvallis, OR, 97331, USA
SOURCE: Plant Lipid Metab., [Pap. Int. Meet. Plant Lipids], 11th (1995), Meeting Date 1994, 499-502. Editor(s): Kader, Jean-Claude; Mazliak, Paul. Kluwer: Dordrecht, Neth.
CODEN: 61OZAO
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Cuphea **mutants** displaying altered seed oil fatty acid profiles have been isolated following EMS **mutagenesis**. The phenotypes of single and double homozygous **mutants** are discussed. CDNA clones encoding putative acyl-ACP thioesterases and .beta.-ketoacyl synthases have been isolated from a C. wrightii embryo library.

L5 ANSWER 45 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:555955 CAPLUS
DOCUMENT NUMBER: 119:155955
TITLE: The presence of a novel type of surface polysaccharide in Rhizobium meliloti requires a new fatty acid synthase-like gene cluster involved in symbiotic nodule development
AUTHOR(S): Petrovics, Gyorgy; Putnoky, Peter; Reuhs, Bradley; Kim, John; Thorp, Tina A.; Noel, K. Dale; Carlson, Russell W.; Kondorosi, Adam
CORPORATE SOURCE: Inst. Genet., Hung. Acad. Sci., Szeged, H-6701, Hung.
SOURCE: Mol. Microbiol. (1993), 8(6), 1083-94
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Bacterial exopolysaccharide (EPS) and lipopolysaccharide (LPS) mols. have been shown to play important roles in **plant**-bacterium interactions. Here, the authors demonstrate that the fix-23 loci, which compensate for exo **mutations** during symbiotic nodule development, are involved in the prodn. of a novel polysaccharide that is rich in 3-deoxy-D-manno-2-octulosonic acid (Kdo) but is not the classical LPS. This mol. is likely to be a surface antigen,since antiserum to whole Rhizobium meliloti cells reacts strongly with it and since **mutations** in fix-23 result in an inability to produce this polysaccharide and to bind bacteriophage 16-3. It is likely that this Kdo-rich polysaccharide is analogous to certain Escherichia **coli** K antigens which are anchored to the membrane via a phospholipid moiety. DNA sequence anal. of one gene cluster of this region revealed that the predicted protein products of six genes exhibit a high degree of homol. and similar organization to those of the rat fatty acid synthase multifunctional enzyme domains.

L5 ANSWER 46 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:229220 CAPLUS
DOCUMENT NUMBER: 116:229220
TITLE: The subcloning and expression of midecamycin polyketide **condensing enzyme** gene

AUTHOR(S): Zhu, Xuewei; Wang, Yiguang; Jin, Lianfang; Xu, Xiaoming
CORPORATE SOURCE: Inst. Med. Biotechnol., Chin. Acad. Med. Sci., Beijing, Peop. Rep. China
SOURCE: Shengwu Gongcheng Xuebao (1991), 7(4), 291-9
CODEN: SGXUED; ISSN: 1000-3061
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The midecamycin polyketide **condensing enzyme** gene derived from cloned DNA pCN8B12 in genomic library of midecamycin-producing strain Streptomyces mycarofaciens 1748 was subcloned onto the Escherichia **coli**-Streptomyces shuttle vector pWHM3. A recombinant plasmid DNA pCG2 was obtained and introduced into an ActI-**mutant** of actinorhodin producer S. coelicolor TK17, the transformants contg. the recombinant plasmid produced a new hybrid antibiotic which was different from midecamycin and actinorhodin by paper chromatog. anal. The transformants of pCG2 DNA in S. ambofaciens showed increased prodn. of spiramycin. The transformants of pCG2 DNA in a regulatory **mutant** of daunorubicin producer S. peucetius H6101 produced a daunorubicin intermediate .epsilon.-rhodomycinone, according to TLC and HPLC anal. The tetracenomycin producer S. glaucescens contg. pCG2 DNA also exhibited certain antibacterial activity, but transformants of pCG2 DNA in Saccharapolyspora erythraea WMH 15,261 showed no antibacterial activity. Presumably, the pCG2 DNA showed a regulatory function or ability to restore antibiotic productivity in certain polyketide synthase deficient **mutants**.

L5 ANSWER 47 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:627977 CAPLUS
DOCUMENT NUMBER: 115:227977
TITLE: Overproduction and localization of components of the polyketide synthase of Streptomyces glaucescens involved in the production of the antibiotic tetracenomycin C
AUTHOR(S): Gramajo, Hugo C.; White, Janet; Hutchinson, C. Richard; Bibb, Mervyn J.
CORPORATE SOURCE: John Innes Cent. Plant Sci. Res., John Innes Inst., Norwich, NR4 7UH, UK
SOURCE: J. Bacteriol. (1991), 173(20), 6475-83
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Three proteins, including the .beta.-keto acyl synthase and the acyl carrier protein, involved in the synthesis of the polyketide antibiotic tetracenomycin C by S. glaucescens GLA.0 were produced in E. **coli** by using the T7 RNA polymerase-dependent pT7-7 expression vector. Changing the N-terminal codon usage of 2 of the genes greatly increased the level of protein produced without affecting mRNA levels, suggesting improvements in translational efficiency. Western immunoblot anal. of cytoplasmic and membrane fractions of S. glaucescens with antibodies raised to synthetic oligopeptides corresponding to the 2 presumed components of the .beta.-keto acyl synthase indicated that both proteins were membrane-bound; 1 could be detected in stationary-phase cultures but not in rapidly growing cultures, correlating with the time of appearance of tetracenomycin C in the medium.

L5 ANSWER 48 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1982:452647 CAPLUS
DOCUMENT NUMBER: 97:52647
TITLE: Studies on **plant** breeding and genetics by radiation application
AUTHOR(S): Kwon, S. H.; Chung, K. H.; Won, J. L.; Oh, J. H.; Kim, J. R.; Shin, I. C.
CORPORATE SOURCE: Korea At. Energy Res. Inst., Seoul, S. Korea
SOURCE: Report (1980), KAERI-395/RR-128/80, 124 pp. Avail.: NTIS (U.S. Sales Only)
From: Energy Res. Abstr. 1982, 7(6), Abstr. No. 15926
DOCUMENT TYPE: Report
LANGUAGE: Korean

AB Radiation **mutagenesis** was used to improve legumes, barley, and vegetatively propagated plants. Some promising **mutants** were selected from the M2 population of irradiated soybean varieties **KAS** 320-4 and CB-27. Fourteen high-yielding **mutants** were selected from the progenies of the Clark variety treated with EMS. The **mutation** frequency in soybean, based on chlorophyll deficiency, was 1% in the variety CB-27. Selections were also for made early maturing and abundant podding in M2 generations of .gamma.-irradiated mung beans, and for cold tolerance, early maturing, and short **plant** height in M3 generations of .gamma.-irradiated naked barley. Treatment for 14 days at the flowering stage was effective in testing soybean tolerance for shading and flooding. For enhanced reprodn. and physiol. studies a technique of grafting soybean plants and reprodn.

by cuttings was developed. In breeding for disease resistance, 1273 **mutant** lines were selected from the M2 population of the soybean varieties Kwangkyo and Kangrim. Of the hybrids between KEX-2 and several soybean varieties, 95 lines were selected for high yielding potential. The promising lines selected from the germ plasm collection, and irradiated and hybrid progenies were evaluated for regional adaptability and 10 lines were selected. The soybean gene pool consists of 2017 native lines and some desirable lines were found in the collections of 1976-1979.

L5 ANSWER 49 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1973:156439 CAPLUS
DOCUMENT NUMBER: 78:156439
TITLE: Synthesis of unsaturated fatty acids and the lesion in fab B **mutants**
AUTHOR(S): Rosenfeld, Ira S.; D'Agnolo, Giuliano; Vagelos, P. Roy
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, Mo., USA
SOURCE: J. Biol. Chem. (1973), 248(7), 2452-60
CODEN: JBCHA3
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two classes of Escherichia **coli** unsatd. fatty acid auxotrophs, which are complementary both in vivo and in vitro, were utilized to study unsatd. fatty acid synthesis. .beta.-Hydroxydecanoyl thioester dehydrase, which catalyzes the 1st committed reaction in the unsatd. fatty acid synthetic pathway, is defective in 1 class, fab A. The defect in the 2nd class, fab B, was unknown. The unsatd. fatty acid synthetic activity defective in fab B exts. was isolated from fab A exts. on the basis of an in vitro complementation assay. Throughout a 586-fold purifn. procedure the fab A unsatd. fatty acid synthetic activity was assocd. with .beta.-**ketoacyl acyl carrier protein** (ACP) synthetase, suggesting that the 2 activities are catalyzed by the same protein. Further support for this suggestion derived from the findings that both activities were purified together during the isolation of homogeneous .beta.-**ketoacyl-ACP synthetase** from wild-type E. **coli** B, both were inhibited to the same extent by iodoacetamide, both were protected against iodo-acetamide inhibition by acetyl-ACP, and both were inactivated at 43.degree. at identical rates. The fact that wild-type .beta.-**ketoacyl-ACP synthetase** stimulated unsatd. fatty acid synthesis by fab B exts. suggested that fab B .beta.-**ketoacyl-ACP synthetase** is defective in unsatd. fatty acid synthetic activity. Although partially purified fab B .beta.-**ketoacyl-ACP synthetase** was shown to be defective in unsatd. fatty acid synthetic activity, the **mutant** enzyme catalyzed normally condensation reactions involving fatty acyl-ACP's that are intermediates in the synthesis of satd. and unsatd. fatty acids. Although the unsatd. fatty acid synthetic activity of .beta.-**ketoacyl-ACP synthetase** is not understood, these studies demonstrate that this enzyme catalyzes a unique reaction in the synthesis of unsatd. fatty acids.

L5 ANSWER 50 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1972:548655 CAPLUS
DOCUMENT NUMBER: 77:148655
TITLE: Acyl carrier protein. XVI. Intermediate reactions of unsaturated fatty acid synthesis in Escherichia **coli** and studies of fab B **mutants**
AUTHOR(S): Birge, Claire H.; Vagelos, P. Roy
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, Mo., USA
SOURCE: J. Biol. Chem. (1972), 247(16), 4921-9
CODEN: JBCHA3
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Synthesis of cis-5-.beta.-hydroxyl-[2-14C]-dodecenoyl-acyl carrier protein (ACP), a postulated intermediate in unsatd. fatty acid synthesis in E. **coli**, was achieved from cis-3-decenoyl-ACP and [2-14C]-malonyl-ACP through the combined action of two enzymes, .beta.-**ketoacyl-ACP synthetase** and .beta.-keto-acyl-ACP reductase. The conversion of cis-5-.beta.-hydroxyl-[2-14C]-dodecenoyl-ACP to cis-5-trans-2-[2-14C]-dodecadienoyl-ACP and to cis-5-[2-14C]-dodecenoyl-ACP was shown utilizing a crude fattv acid synthetase prepn. of wild type E. **coli**. Elongation of cis-5-.beta.-hydroxyl-[2-14C]-dodecenoyl-ACP to long-chain unsatd. fatty acids was also shown with this fatty acid synthetase prepn. These studies indicate that cis-5-.beta.-hydroxydodecenoyl-ACP, cis-5-trans-2-dodecadienoyl-ACP, and cis-5-dodecenoyl-ACP are intermediates in the synthesis of long-chain unsatd. fatty acids. Exts. of a class of fatty acid biosynthetic **mutants**, fab B, which specifically cannot synthesize unsatd. fatty acids although synthesizing satd. fatty acids normally, were examd. for all the above activities. No apparent defect was revealed in any step involved in the utilization of cis-5-.beta.-hydroxy-[2-14C]-dodecenoyl-ACP for long-chain unsatd. fatty acid synthesis. **Mutant** exts. were

also found to contain normal amts. of .beta.-ketoacyl-ACP reductase and .beta.-hydroxyacyl-ACP dehydrase, which apparently function in both the satd. and unsatd. fatty acid biosynthetic pathways, and of enoyl-ACP reductase. It is concluded that fab B **mutants** contain a **mutation** in an enzyme which catalyzes a reaction unique to the unsatd. fatty acid pathway which is yetunknown.

L5 ANSWER 51 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1965:61066 CAPLUS
DOCUMENT NUMBER: 62:61066
ORIGINAL REFERENCE NO.: 62:10870g-h,10871a
TITLE: Regulatory mechanisms in the biosynthesis of isoleucine and valine. II. Identification of two operator genes
AUTHOR(S): Ramakrishnan, T.; Adelberg, Edward A.
CORPORATE SOURCE: Yale Univ.
SOURCE: J. Bacteriol. (1965), 89(3), 654-60
DOCUMENT TYPE: Journal
LANGUAGE: English
AB cf. CA 60, 9648e. A tightly clustered set of 5 structural genes governs the synthesis of the 5 enzymes of isoleucine and valine biosynthesis in Escherichia **coli**. Three of the genes, governing transaminase B, dehydrase, and threonine deaminase, are controlled by a single operator locus, designated oprA. The structural gene governing the **condensing enzyme** is controlled by a 2nd operator locus, designated oprB. Both oprA and oprB have been shown to regulate structural genes which are, cis but not trans, to their own operator. No **mutations** have yet been found which affect the level of reductoisomerase, but the existence of a 3rd operator controlling the synthesis of this enzyme can be inferred. Enzyme derepression resulting from **mutations** in oprA confers resistance to high levels of valine. Derepression of the **condensing enzyme** resulting from **mutations** in oprB confers resistance to low levels of valine, and to .alpha.-aminobutyric acid. The significance of these findings with respect to the valine sensitivity of E. **coli** strain K-12 is discussed.

L5 ANSWER 52 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1964:54622 CAPLUS
DOCUMENT NUMBER: 60:54622
ORIGINAL REFERENCE NO.: 60:9648e-f
TITLE: Regulatory mechanisms in the biosynthesis of isoleucine and valine. I. Genetic derepression of enzyme formation
AUTHOR(S): Ramakrishnan, T.; Adelberg, Edward A.
CORPORATE SOURCE: Yale Univ.
SOURCE: J. Bacteriol. (1964), 87(3), 566-73
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB A total of 60 **mutants** of Escherichia **coli** K-12 resistant to 10-2M valine were isolated from the valine-sensitive F' strain AB1206. Conjugation expts. showed that in 5 of these **mutants** the valine-resistance locus is closely linked to the structural genes governing isoleucine-valine biosynthesis. In these 5 valine-resistant **mutants**, 3 enzymes of the isoleucine-valine pathway were found to be coordinately derepressed; L-threonine deaminase, dihydroxy acid dehydrase, and transaminase B. Two other enzymes of this pathway, the **condensing enzyme** and the reductoisomerase, were unaffected. The **mutation** from valine-sensitivity to valine-resistance appears to have altered an operator locus, because the derepressed state is dominant over the repressed state in diploids heterozygous for the valine-resistance locus. The valine-resistant **mutants** excrete isoleucine into the medium. The significance of these findings with respect to the valine-sensitivity of E. **coli** K-12 and the regulation of the biosynthesis of isoleucine and valine by this organism are discussed.

L5 ANSWER 53 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1962:432055 CAPLUS
DOCUMENT NUMBER: 57:32055
ORIGINAL REFERENCE NO.: 57:6437a-f
TITLE: Selective utilization of metabolic rouses by Escherichia **coli**
AUTHOR(S): Kornberg, H. L.
CORPORATE SOURCE: Univ. Oxford, UK
SOURCE: Cold Spring Harbor Symp. Quant. Biol. (1961), 26, 257-9
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB In sonic exts. of E. **coli** the levels of various key enzymes of carbohydrate utilization by different pathways are profoundly affected by the nature of the growth substrate used. When lactate-grown cells are

placed in a medium contg. acetate as sole C source, the sp. activities of the enzymes of glyoxalate cycle rapidly rises 4-6-fold before growth can be detected, and rises more slowly thereafter to the levels which are maintained const. during growth on acetate, while the enzymes of glycerate pathway are not increased in activity. Lactate-grown cells placed in media contg. glycolate as the sole C source rapidly form the key enzymes of the glycerate pathway before growth is detected. While acetate-grown cells continuing to grow on acetate continue to form large amts. of isocitratase but only small amts. of tartronic semialdehyde reductase, such cells continuing to grow on glycolate synthesize only small amts. of isocitratase but large amts. of the reductase. Glycolate-grown cells continuing to grow on glycolate continue to form large amts. of glyoxylate carboligase and tartronic semialdehyde reductase, while such cells placed in acetate growth media grow but do not continue to form these enzymes in significant amts. Analysis of sonic exts. of **mutant** strain M22-64, which lacks the **condensing enzyme** and which is incapable of utilizing acetate unless glycolate or glyoxylate is added, show that this addn. of acetate is followed by the almost total cessation of synthesis of glyoxylate carboligase. Growth, however, is not affected and no increased amts. of isocitratase are produced. Similarly, when the **mutant** grows on a medium contg. small amts. of acetate plus an excess of glycolate, glyoxylate carboligase is formed only after the acetate is used up, but thereafter is produced at a rate similar to that noted with a culture that has not received acetate. When this strain grows on glutamate as the sole C source, isocitratase is synthesized at rates entirely unaffected by the addn. of acetate. These observations illustrate that organisms genetically capable of forming specific enzymes are capable of so doing under a wide variety of environmental conditions, but may not fully express this genetic capability except when required for the survival of the organism, and that the selective formation of key enzymes of unique metabolic routes enables the organism to adjust to changes in their nutritional environment with efficiency and economy, and that these highly specific phenotypic changes may be brought about by the relief of enzyme repression.

L5 ANSWER 54 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1962:55913 CAPLUS
DOCUMENT NUMBER: 56:55913
ORIGINAL REFERENCE NO.: 56:10682d-h
TITLE: Metabolism of C2-compounds in microorganisms. VIII. A dicarboxylic acid cycle as a route for the oxidation of glycolate by Escherichia **coli**
AUTHOR(S): Kornberg, H. L.; Sadler, J. R.
CORPORATE SOURCE: Univ. Oxford, UK
SOURCE: Biochem. J. (1961), 81, 503-13
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. CA 54, 11135b; 56, 5100d. E. **coli** strain w grew readily in a medium contg. 50 mM glycolate as C source. The **mutant** M22-64 (Gilvarg and Davis, J. Biol. Chem. 222, 307-19(1957)), devoid of the citrate-forming **condensing enzyme**, failed to grow in this medium unless small quantities (2mM) of glutamate were also added. The **mutant** and wild-type organisms, grown on 55 mM glycolate plus 2 mM glutamate, oxidized glycolate or glyoxyl ate at the same rate and to the same extent. These oxidns. were not affected by 2.5 mM hypophosphite, which abolished formate oxidn. Isotope from glycolate-1-C14 or -2-C14 was incorporated rapidly, by organisms oxidizing unlabeled glycolate, into glyoxylate, malate, oxaloacetate, and pyruvote. Under these conditions, C14 from acetate-2-C14 appeared in malate, oxaloacetate, and pyruvate. Although the **mutant** did not oxidize acetate, acetate stimulated the rate and extent of O uptake by cells oxidizing glyoxylate. Washed suspensions of the **mutant** evolved, as C14O2, 0.5% of the isotope from acetate-1-C14. This was increased 70-80 fold when unlabeled glycolate or glyoxylate was added. Pyruvate was ineffective in promoting this oxidn. of acetate-1-C14. Similar results were obtained with acetate-2-C14. Addn. of unlabeled glycolate or glyoxylate stimulated the release of C14 as C14O2 from malate-1-C14, malate-2,3- C14 and pyruvate-3-C14. These results suggest the operation of a dicarboxylic acid cycle, in which glyoxylate is totally oxidized with the catalytic participation of malate, oxaloacetate, pyruvate, and acetyl coenzyme A. Measurement of the abs. levels of enzymes (glycolic acid oxidase, malate synthetase, malate dehydrogenase, oxaloacetate decarboxylase, pyruvate oxidase, and fumarase) in glycolate-grown cells, and alterations in their relative proportions when cells adapt to grow on glycolate, support the occurrence of this postulated cycle.

L5 ANSWER 55 OF 57 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1956:90121 CAPLUS
DOCUMENT NUMBER: 50:90121
ORIGINAL REFERENCE NO.: 50:16979h-i,16980a
TITLE: The role of the tricarboxylic acid cycle in acetate oxidation in Escherichia **coli**

AUTHOR(S): Gilvarg, Charles; Davis, Bernard D.
CORPORATE SOURCE: Cornell Univ. Med. Coll., New York, NY
SOURCE: J. Bid. Chem. (1956), 222, 307-19
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 44, 9512c. A no. of **mutants** of E. **coli** and Aerobacter aerogenes lack the citrate-**condensing enzyme** ; there is reason to believe that no other reaction is blocked in these strains. As a result of the metabolic block, these **mutants** exhibit (a) a nutritional requirement for glutamate or .alpha.-ketoglutarate, (b) a virtually complete loss of the ability to oxidize acetate (but not succinate), and (c) a considerable decrease in the extent to which glucose is oxidized to CO2. These findings offer strong evidence that the tricarboxylic acid cycle is the only significant pathway in these species for acetate oxidation, as well as for .alpha.-ketoglutarate synthesis. Succinate formation is excluded. These findings suggest that alternative pathways, not involving acetate, do not play a major part in the oxidation of glucose in these species.

L5 ANSWER 56 OF 57 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:331692 BIOSIS
DOCUMENT NUMBER: PREV199497344692
TITLE: Characterization of a novel form of Escherichia **coli** acyl carrier protein associated with **mutants** lacking 3-**ketoacyl-ACP synthase** II.
AUTHOR(S): Keating, D.; Cronan, J., Jr.
CORPORATE SOURCE: Univ. Ill., Urbana, IL USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1994) Vol. 94, No. 0, pp. 290. Meeting Info.: 94th General Meeting of the American Society for Microbiology Las Vegas, Nevada, USA May 23-27, 1994 ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L5 ANSWER 57 OF 57 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1987:5335 BIOSIS
DOCUMENT NUMBER: BA83:5335
TITLE: COLON CARCINOMA K-RAS 2 ONCOGENE OF A FAMILIAL POLYPOSIS **COLI** PATIENT.
AUTHOR(S): YUASA Y; OTO M; SATO C; MIYAKI M; IWAMA T; TONOMURA A; NAMBA M
CORPORATE SOURCE: DEP. HYG., GUNMA UNIV. SCH. MED., 39-22 SHOWA-MACHI 3-CHOME, MAEBASHI 371, JPN.
SOURCE: JPN J CANCER RES (GANN), (1986) 77 (9), 901-907. CODEN: JJCREP. ISSN: 0910-5050.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The DNA of a colon carcinoma-derived cell line (KMS-4) and that of skin fibroblasts from a familial polyposis **coli** patient were transfected into NIH3T3 cells in order to detect oncogenes associated with the disease. No transformation was observed with the normal skin fibroblast DNA, while the KMS-4 cell DNA was able to transform NIH3T3 cells. Through hybridization with known oncogene probes, the KMS-4 transforming gene was found to be a human activated c-K-ras 2 oncogene. Sequence analysis of the molecularly cloned KMS-4 c-K-ras 2 oncogene showed a single nucleotide transition from G to T at the 12th codon. This results in substitution of cysteine for glycine at this position. On using labeled synthetic oligonucleotides to detect the **mutation** in codon 12, we found the G to T transition in colon carcinoma cells. This suggests that activation of the c-**Kas**-ras 2 oncogene could be associated with colon carcinoma induction.

CONTINUE? Y/(N):y

L7 ANSWER 1 OF 17 MEDLINE
ACCESSION NUMBER: 1999196473 MEDLINE
DOCUMENT NUMBER: 99196473 PubMed ID: 10098837
TITLE: Cloning and characterization of zRICH, a 2',3'-cyclic-nucleotide 3'-phosphodiesterase induced during zebrafish optic nerve regeneration.
AUTHOR: Ballestero R P; Dybowski J A; Levy G; Agranoff B W; Uhler M D
CORPORATE SOURCE: Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor 48104-1687, USA.
SOURCE: JOURNAL OF NEUROCHEMISTRY, (1999 Apr) 72 (4) 1362-71. Journal code: JAV; 2985190R. ISSN: 0022-3042.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990426
Last Updated on STN: 19990426
Entered Medline: 19990413

AB We previously reported cloning of cDNAs encoding both components of a protein doublet induced during goldfish optic nerve regeneration. The predicted protein sequences showed significant homology with the mammalian 2',3'-cyclic-nucleotide 3'-phosphodiesterases (CNPases). CNPases are well-established markers of mammalian myelin; hence, the cDNAs were designated gRICH68 and gRICH70 (for goldfish Regeneration-Induced CNPase Homologues of 68 and 70 kDa). Homologous cDNAs have now been isolated from zebrafish encoding a highly related protein, which we have termed zRICH. RNase protection assays show that zRICH mRNA is induced significantly (fivefold) in optic nerve regenerating zebrafish retinas 7 days following nerve crush. Western blots show a single band in zebrafish brain and retina extracts, with immunoreactivity increasing three-fold in regenerating retinas 21 days postcrush. Immunohistochemical analysis indicated that this increase in zRICH protein expression is localized to the retinal ganglion cell layer in regenerating retina. We have characterized and evaluated the relevance of a conserved beta-**ketoacyl synthase** motif in zRICH to CNPase activity by means of site-directed **mutagenesis**. Two residues within the motif, H334 and T336, are critical for enzymatic activity. A cysteine residue within the motif, which corresponds to a critical residue for beta-**ketoacyl synthase**, does not appear to participate in the phosphodiesterase activity.

L7 ANSWER 2 OF 17 MEDLINE
ACCESSION NUMBER: 1998345981 MEDLINE
DOCUMENT NUMBER: 98345981 PubMed ID: 9681003
TITLE: Condensing enzymes from Cuphea wrightii associated with medium chain fatty acid biosynthesis.
AUTHOR: Slabaugh M B; Leonard J M; Knapp S J
CORPORATE SOURCE: Department of Crop and Soil Science, Oregon State University, Corvallis 97331, USA.. slabaugm@css.orst.edu
SOURCE: PLANT JOURNAL, (1998 Mar) 13 (5) 611-20. Journal code: BRU; 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U67316; GENBANK-U67317
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980817

AB Seed oils of most Cuphea species contain > 90% medium chain (C8-C14) fatty acids. Thioesterases with **specificity** for these substrates are important determinants of the medium chain phenotype. The role of condensing enzymes, however, has not been investigated. cDNA clones encoding beta-**ketoacyl-acyl carrier protein** (ACP) synthase (**KAS**) were isolated from C. wrightii, a C10/C12-producing species. Deduced amino acid sequences of four unique clones were approximately 60% identical to plant **KAS** I sequences and approximately 75% identical to a distinct class of **KAS** sequences recently identified in castor and barley. A 46 kDa protein that was observed only in developing and mature seed was detected using antiserum directed against recombinant Cuphea **KAS** protein. The 46 kDa protein was abundant in developing seeds of six medium chain-producing Cuphea species but barely detected in one long chain-producing species. A 48 kDa protein identified immunologically as **KAS** I was expressed in both medium and long chain-producing Cuphea species and was detected in all tissues tested. In in vitro assays,

extracts from C. wrightii and C. viscosissima developing embryos were unable to extend fatty acid chains beyond C10 following treatment with 10 microns cerulenin, a potent inhibitor of **KAS** I. However, a C. viscosissima **mutant**, cpr-1, whose seed oils are deficient in caprate relative to wild type, was impaired in extension of C8 to C10 in this assay and Western analysis revealed a specific deficiency in 46 kDa **KAS** in cpr-1 embryos. These results implicate cerulenin-resistant condensing activity in production of medium chain fatty acids in Cuphea.

L7 ANSWER 3 OF 17 MEDLINE
ACCESSION NUMBER: 1998070260 MEDLINE
DOCUMENT NUMBER: 98070260 PubMed ID: 9405069
TITLE: Characterization of the interthiol acyltransferase reaction catalyzed by the beta-**ketoacyl synthase** domain of the animal fatty acid synthase.
AUTHOR: Witkowski A; Joshi A K; Smith S
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, California 94609, USA.
CONTRACT NUMBER: DK 16073 (NIDDK)
SOURCE: BIOCHEMISTRY, (1997 Dec 23) 36 (51) 16338-44.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980130
Last Updated on STN: 20000303
Entered Medline: 19980122

AB The enzyme activity responsible for translocation of saturated acyl chains from the 4'-phosphopantetheine of the acyl carrier protein to the active site cysteine of the beta-**ketoacyl synthase** in the animal fatty acid synthase has been identified. An enzyme assay was devised that allows uncoupling of the interthiol transfer step from the condensation reaction. Experiments with various fatty acid synthase **mutants** indicate clearly that catalysis of the transfer of saturated acyl moieties from the 4'-phosphopantetheine thiol to the active site cysteine thiol, Cys-161, is an inherent property of the beta-**ketoacyl synthase** domain. Catalytic efficiency of the interthiol transferase increases from C2 to C12 and decreases with increasing chain-lengths beyond C12. Malonyl, beta-hydroxybutyryl, and crotonyl thioesters are not substrates for the transferase, whereas the beta-ketobutyryl moiety is a poor substrate. These features of the substrate **specificity** are exactly as predicted for a transferase that fulfills the proposed role in the fatty acid synthase reaction sequence and indicate that this activity plays an important role in determining the overall **specificity** of the beta-**ketoacyl synthase** reaction.

L7 ANSWER 4 OF 17 MEDLINE
ACCESSION NUMBER: 1998042824 MEDLINE
DOCUMENT NUMBER: 98042824 PubMed ID: 9375254
TITLE: Molecular recognition of diketide substrates by a beta-**ketoacyl-acyl carrier protein** synthase domain within a bimodular polyketide synthase.
AUTHOR: Chuck J A; McPherson M; Huang H; Jacobsen J R; Khosla C; Cane D E
CORPORATE SOURCE: Department of Chemistry, Brown University, Providence, RI 02912-9108, USA.
CONTRACT NUMBER: GM22172 (NIGMS)
SOURCE: CHEMISTRY AND BIOLOGY, (1997 Oct) 4 (10) 757-66.
Journal code: CNA; 9500160. ISSN: 1074-5521.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971222

AB BACKGROUND: Modular polyketide synthases (PKSs) are large multifunctional proteins that catalyze the biosynthesis of structurally complex bioactive products. The modular organization of PKSs has allowed the application of a combinatorial approach to the synthesis of novel polyketides via the manipulation of these biocatalysts at the genetic level. The inherent **specificity** of PKSs for their natural substrates, however, may place limits on the spectrum of molecular diversity that can be achieved in polyketide products. With the aim of further understanding PKS **specificity**, as a route to exploiting PKSs in combinatorial synthesis, we chose to examine the substrate **specificity** of a single intact domain within a bimodular PKS to investigate its capacity to

utilize unnatural substrates. RESULTS: We used a blocked **mutant** of a bimodular PKS in which formation of the triketide product could occur only via uptake and processing of a synthetic diketide intermediate. By introducing systematic changes in the native diketide structure, by means of the synthesis of unnatural diketide analogs, we have shown that the ketosynthase domain of module 2 (KS2 domain) in 6-deoxyerythronolide B synthase (DEBS) tolerates a broad range of variations in substrate structure, but it strongly discriminates against some others. CONCLUSIONS: Defining the boundaries of substrate recognition within PKS domains is crucial to the rationally engineered biosynthesis of novel polyketide products, many of which could be prepared only with great difficulty, if at all, by direct chemical synthesis or semi-synthesis. Our results suggest that the KS2 domain of DEBS1 has a relatively relaxed **specificity** that can be exploited for the design and synthesis of medicinally important polyketide products.

L7 ANSWER 5 OF 17 MEDLINE
ACCESSION NUMBER: 97252499 MEDLINE
DOCUMENT NUMBER: 97252499 PubMed ID: 9098068
TITLE: Minimal Streptomyces sp. strain C5 daunorubicin polyketide biosynthesis genes required for aklanonic acid biosynthesis.
AUTHOR: Rajgarhia V B; Strohl W R
CORPORATE SOURCE: Department of Microbiology, The Ohio State University, Columbus 43210, USA.
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Apr) 179 (8) 2690-6.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970523
Last Updated on STN: 19970523
Entered Medline: 19970509

AB The structure of the Streptomyces sp. strain C5 daunorubicin type II polyketide synthase (PKS) gene region is different from that of other known type II PKS gene clusters. Directly downstream of the genes encoding **ketoacylsynthase** alpha and beta (KS alpha, KS beta) are two genes (dpsC, dpsD) encoding proteins of unproven function, both absent from other type II PKS gene clusters. Also in contrast to other type II PKS clusters, the gene encoding the acyl carrier protein (ACP), dpsG, is located about 6.8 kbp upstream of the genes encoding the daunorubicin KS alpha and KS beta. In this work, we demonstrate that the minimal genes required to produce aklanonic acid in heterologous hosts are dpsG (ACP), dauI (regulatory activator), dpsA (KS alpha), dpsB (KS beta), dpsF (aromatase), dpsE (polyketide reductase), and dauG (putative deoxyaklanonic acid oxygenase). The two unusual open reading frames, dpsC (KASIII homolog lacking a known active site) and dpsD (acyltransferase homolog), are not required to synthesize aklanonic acid. Additionally, replacement of dpsD or dpsCD in Streptomyces sp. strain C5 with a neomycin resistance gene (aphI) results in **mutant** strains that still produced anthracyclines.

L7 ANSWER 6 OF 17 MEDLINE
ACCESSION NUMBER: 96351420 MEDLINE
DOCUMENT NUMBER: 96351420 PubMed ID: 8742713
TITLE: A jojoba beta-Ketoacyl-CoA synthase cDNA complements the canola fatty acid elongation **mutation** in transgenic plants.
AUTHOR: Lassner M W; Lardizabal K; Metz J G
CORPORATE SOURCE: Calgene, Inc., Davis, California 95616, USA.
SOURCE: PLANT CELL, (1996 Feb) 8 (2) 281-92.
Journal code: BJU; 9208688. ISSN: 1040-4651.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U37088
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961025
Last Updated on STN: 19980206
Entered Medline: 19961011

AB beta-Ketoacyl-coenzyme A (CoA) synthase (KCS) catalyzes the condensation of malonyl-CoA with long-chain acyl-CoA. This reaction is the initial step of the microsomal fatty acyl-CoA elongation pathway responsible for formation of very long chain fatty acids (VLCFAs, or fatty acids with chain lengths > 18 carbons). Manipulation of this pathway is significant for agriculture, because it is the basis of conversion of high erucic acid rapeseed into canola. High erucic acid rapeseed oil, used as an industrial feedstock, is rich in VLCFAs, whereas the edible oil extracted from canola is essentially devoid of VLCFAs. Here, we report the cloning of a cDNA

from developing jojoba embryos involved in microsomal fatty acid elongation. The jojoba cDNA is homologous to the recently cloned Arabidopsis FATTY ACID ELONGATION1 (FAE1) gene that has been suggested to encode KCS. We characterize the jojoba enzyme and present biochemical data indicating that the jojoba cDNA does indeed encode KCS. Transformation of low erucic acid rapeseed with the jojoba cDNA restored KCS activity to developing embryos and altered the transgenic seed oil composition to contain high levels of VLCFAs. The data reveal the key role KCS plays in determining the chain lengths of fatty acids found in seed oils.

L7 ANSWER 7 OF 17 MEDLINE
ACCESSION NUMBER: 96064736 MEDLINE
DOCUMENT NUMBER: 96064736 PubMed ID: 7592873
TITLE: Enoyl-acyl carrier protein reductase (fabI) plays a determinant role in completing cycles of fatty acid elongation in Escherichia coli.
AUTHOR: Heath R J; Rock C O
CORPORATE SOURCE: Department of Biochemistry, St Jude Children's Research Hospital, Memphis, Tennessee 38101, USA.
CONTRACT NUMBER: CA 21765 (NCI)
GM34496 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 3) 270 (44) 26538-42.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951221

AB The role of enoyl-acyl carrier protein (ACP) reductase (E.C. 1.3.1.9), the product of the fabI gene, was investigated in the type II, dissociated, fatty acid synthase system of Escherichia coli. All of the proteins required to catalyze one cycle of fatty acid synthesis from acetyl-CoA plus malonyl-CoA to butyryl-ACP in vitro were purified. These proteins were malonyl-CoA:ACP transacylase (fabD), beta-**ketoacyl-ACP synthase** III (fabH), beta-ketoacyl-ACP reductase (fabG), beta-hydroxydecanoyl-ACP dehydrase (fabA), and enoyl-ACP reductase (fabI). Unlike the other enzymes in the cycle, FabA did not efficiently convert its substrate beta-hydroxybutyryl-ACP to crotonyl-ACP, but rather the equilibrium favored formation of beta-hydroxybutyryl-ACP over crotonyl-ACP by a ratio of 9:1. The amount of butyryl-ACP formed depended on the amount of FabI protein added to the assay. Extracts from fabI(Ts) **mutants** accumulated beta-hydroxybutyryl-ACP, and the addition of FabI protein to the fabI(Ts) extract restored both butyryl-ACP and long-chain acyl-ACP synthesis. FabI was verified to be the only enoyl-ACP reductase required for the synthesis of fatty acids by demonstrating that purified FabI was required for the elongation of both long-chain saturated and unsaturated fatty acids. These results were corroborated by analysis of the intracellular ACP pool composition in fabI(Ts) **mutants** that showed beta-hydroxybutyryl-ACP and crotonyl-ACP accumulated at the nonpermissive temperature in the same ratio found in the fabI(Ts) extracts and in the in vitro reconstruction experiments that lacked FabI. We conclude that FabI is the only enoyl-ACP reductase involved in fatty acid synthesis in E. coli and that the activity of this enzyme plays a determinant role in completing cycles of fatty acid biosynthesis.

L7 ANSWER 8 OF 17 MEDLINE
ACCESSION NUMBER: 95014072 MEDLINE
DOCUMENT NUMBER: 95014072 PubMed ID: 7928998
TITLE: Isolation and sequence analysis of polyketide synthase genes from the daunomycin-producing Streptomyces sp. strain C5.
AUTHOR: Ye J; Dickens M L; Plater R; Li Y; Lawrence J; Strohl W R
CORPORATE SOURCE: Department of Microbiology, Ohio State University, Columbus 43210-1292.
CONTRACT NUMBER: 43345
SOURCE: JOURNAL OF BACTERIOLOGY, (1994 Oct) 176 (20) 6270-80.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L34880
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941110

AB A contiguous region of about 30 kbp of DNA putatively encoding reactions in daunomycin biosynthesis was isolated from Streptomyces sp. strain C5

DNA. The DNA sequence of an 8.1-kbp EcoRI fragment, which hybridized with actI polyketide synthase (PKS) and actIII polyketide reductase (PKR) gene probes, was determined, revealing seven complete open reading frames (ORFs), two in one cluster and five in a divergently transcribed cluster. The former two genes are likely to encode PKR and a bifunctional cyclase/dehydrase. The five latter genes encode: (i) a homolog of TcmH, an oxygenase of the tetracenomycin biosynthesis pathway; (ii) a PKS Orf1 homolog; (iii) a PKS Orf2 homolog (chain length factor); (iv) a product having moderate sequence identity with Escherichia coli beta-**ketoacyl acyl carrier protein** synthase III but lacking the conserved active site; and (v) a protein highly similar to several acyltransferases. The DNA within the 8.1-kbp EcoRI fragment restored daunomycin production to two dauA non-daunomycin-producing **mutants** of Streptomyces sp. strain C5 and restored wild-type antibiotic production to Streptomyces coelicolor B40 (act VII; nonfunctional cyclase/dehydrase), and to S. coelicolor B41 (actIII) and Streptomyces galilaeus ATCC 31671, strains defective in PKR activity.

L7 ANSWER 9 OF 17 MEDLINE
ACCESSION NUMBER: 94179123 MEDLINE
DOCUMENT NUMBER: 94179123 PubMed ID: 8132481
TITLE: Analysis of type II polyketide beta-**ketoacyl synthase specificity** in Streptomyces coelicolor A3(2) by trans complementation of actinorhodin synthase **mutants**.
AUTHOR: Kim E S; Hopwood D A; Sherman D H
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, St. Paul 55108.
CONTRACT NUMBER: GM-39784 (NIGMS)
GM-46884 (NIGMS)
SOURCE: JOURNAL OF BACTERIOLOGY, (1994 Mar) 176 (6) 1801-4.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940428
Last Updated on STN: 19940428
Entered Medline: 19940421
AB Complementation of defined actinorhodin beta-**ketoacyl synthase** (KS) **mutants** by various other KS genes suggested that the ORF1-encoded KS may be relatively generalized in function, whereas the ORF2-encoded KS component may provide **specificity** in polyketide chain construction. Evidence for differential temporal-spatial expression of the actinorhodin and spore pigment KSs in Streptomyces coelicolor was obtained.

L7 ANSWER 10 OF 17 MEDLINE
ACCESSION NUMBER: 93229805 MEDLINE
DOCUMENT NUMBER: 93229805 PubMed ID: 8471795
TITLE: Interaction of Xanthomonas campestris with Arabidopsis thaliana: characterization of a gene from X. c. pv. raphani that confers avirulence to most A. thaliana accessions.
AUTHOR: Parker J E; Barber C E; Fan M J; Daniels M J
CORPORATE SOURCE: Sainsbury Laboratory, John Innes Centre, Norwich Research Park, U.K.
SOURCE: MOLECULAR PLANT-MICROBE INTERACTIONS, (1993 Mar-Apr) 6 (2) 216-24.
Journal code: A9P; 9107902. ISSN: 0894-0282.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M99059
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930604
Last Updated on STN: 19930604
Entered Medline: 19930520
AB Infiltration of leaves of Arabidopsis thaliana accession Columbia with Xanthomonas campestris pathovar campestris leads to bacterial growth and disease symptoms reminiscent of those incited in Brassica plants inoculated under the same conditions. A search among A. thaliana accessions for variation in the reaction phenotype to strains of X. campestris pathovars campestris, aberrans, and raphani showed that there were no clear differential responses between plant accessions to the individual bacterial strains tested. X. c. pv. raphani strain 1067 was avirulent to all A. thaliana accessions tested. A gene was cloned from X. c. pv. raphani 1067 which, when transferred into the virulent X. c. pv. campestris strain 8004, strongly reduced symptom development and bacterial growth in A. thaliana Columbia plants but did not affect virulence to

Brassica plants. The gene (denoted avrXca) interacted with all A. thaliana accessions tested except one, **Kas**-1, which developed disease symptoms and supported growth of the transconjugant to levels similar to those with X. c. pv. campestris 8004 alone. Sequence analysis of avrXca revealed a probable open reading frame encoding a protein of 66,566 Da that has no homology with other known sequences. A sequence motif conserved among hrp genes was identified in the 5' noncoding region of avrXca, and features characteristic of a signal peptide were found in the N-terminal portion of the presumed AvrXca protein. DNA from different phytopathogenic bacteria contained sequences hybridizing with avrXca in related X. campestris pathovars but not in Erwinia or Pseudomonas strains.

L7 ANSWER 11 OF 17 MEDLINE
ACCESSION NUMBER: 81069887 MEDLINE
DOCUMENT NUMBER: 81069887 PubMed ID: 7002930
TITLE: Structural, enzymatic, and genetic studies of beta-**ketoacyl-acyl carrier protein** synthases I and II of Escherichia coli.
AUTHOR: Garwin J L; Klages A L; Cronan J E Jr
CONTRACT NUMBER: AI 15650 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1980 Dec 25) 255 (24) 11949-56.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198102
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19980206
Entered Medline: 19810219

AB Beta-**Ketoacyl-acyl carrier protein** synthases I and II of Escherichia coli were purified and characterized. Synthase I was shown to have a molecular weight of 80,000 +/- 5,000 and to be composed of two similarly sized subunits. Synthase II had a molecular weight of 85,000 +/- 5,000 and also was apparently homodimeric. Gel electrophoresis of partial proteolytic digests demonstrated that synthases I and II share few if any common peptides. Synthases I and II also were shown to be unrelated by immunological criteria. An improved assay for beta-**ketoacyl-acyl carrier protein** synthase activity gave kinetic parameters for synthases I and II at both 27 degrees C and 37 degrees C using five long chain acyl-acyl carrier protein substrates. The properties of synthase II are consistent with the proposed role of this enzyme in the modulation of fatty acid synthesis by temperature. fabF **mutants** of E. coli lack synthase II. The fabF locus was mapped at min 24.5 of the E. coli genetic map and the clockwise map order was found to be pyrC, fabD, fabF, purB.

L7 ANSWER 12 OF 17 MEDLINE
ACCESSION NUMBER: 80159932 MEDLINE
DOCUMENT NUMBER: 80159932 PubMed ID: 6988423
TITLE: Beta-**ketoacyl-acyl carrier protein** synthase II of Escherichia coli. Evidence for function in the thermal regulation of fatty acid synthesis.
AUTHOR: Garwin J L; Klages A L; Cronan J E Jr
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1980 Apr 25) 255 (8) 3263-5.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198006
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19980206
Entered Medline: 19800625

AB Cvc- **mutants** of Escherichia coli are deficient in the synthesis of cis-vaccenic acid and in the temperature control of fatty acid synthesis. In this communication, it is demonstrated that these **mutants** lack beta-**ketoacyl-acyl carrier protein** synthase II. The deficiencies in cis-vaccenate synthesis and synthase II are shown to be due to a lesion in the same gene, fabF. Lesions in the fabF gene are found to affect growth only when the strain also carries a lesion in the fabB gene, the structural gene for beta-**ketoacyl-acyl carrier protein** synthase I.

L7 ANSWER 13 OF 17 MEDLINE
ACCESSION NUMBER: 75189456 MEDLINE
DOCUMENT NUMBER: 75189456 PubMed ID: 237914
TITLE: Multiple forms of beta-**ketoacyl-acyl**

carrier protein synthetase in Escherichia coli.
AUTHOR: D'Agnolo G; Rosenfeld I S; Vagelos P R
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1975 Jul 25) 250 (14) 5289-94.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197510
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19980206
Entered Medline: 19751010

AB Two forms of beta-**ketoacyl-acyl carrier protein** (ACP) synthetase (designated I and II) have been identified in extracts of Escherichia coli. Synthetase I corresponds to the **condensing enzyme** that was studied earlier (GREENSPAN, M.D., ALBERTS, A.W., and VAGELOS, P.R. (1969) J. Biol. Chem. 244, 6477-6485); synthetase II represents a new form of the enzyme. Synthetase II was isolated as a homogeneous protein. It differs from synthetase I in having a higher molecular weight (76,999 versus 66,000), a lower pH optimum (5.5 to 6.1 versus 7.2), and a greater resistance to denaturation by heat. Synthetase II is similar to synthetase I in that both are inactivated by iodoacetamide, and prior incubation of the enzymes with fatty acyl thioesters prevents the inhibitory effect of iodoacetamide. Both also react with a fatty acyl thioester to form an acyl-enzyme intermediate, and the latter reacts with malonyl-ACP to form a beta-ketoacyl thioester. **Specificity** studies indicated that synthetase II, like synthetase I, has similar affinities with saturated and cis unsaturated fatty acyl thioesters of ACP that are intermediates in the synthesis of saturated and unsaturated fatty acids, respectively. The two synthetases differ only with respect to reactivity with palmitoleyl thioesters: synthetase II has a lower Km and higher Vmax than synthetase I with palmitoleyl-ACP. This finding suggests that synthetase II functions specifically in the elongation of palmitoleyl-ACP to form cis-vaccenyl-ACP. An investigation of synthetases I and II in two classes of unsaturated fatty acid auxotrophs revealed that synthetase I is absent in one class, fabB. Addition of wild type synthetase I to fabB fatty acid synthetase, which synthesizes only saturated fatty acids, permitted this fatty acid synthetase to synthesize unsaturated fatty acids. These experiments indicate that synthetase I plays a critical role in the synthesis of unsaturated fatty acids.

L7 ANSWER 14 OF 17 AGRICOLA
ACCESSION NUMBER: 97:41722 AGRICOLA
DOCUMENT NUMBER: IND20571232
TITLE: **Mutation** or increased copy number of nodE has no effect on the spectrum of chitolipooligosaccharide nod factors made by Rhizobium leguminosarum bv. trifolii.
AUTHOR(S): Philip-Hollingsworth, S.; Orgambide, G.G.; Bradford, J.J.; Smith, D.K.; Hollingsworth, R.I.; Dazzo, F.B.
CORPORATE SOURCE: Michigan State University, East Lansing, MI.
AVAILABILITY: DNAL (381 J824)
SOURCE: The Journal of biological chemistry, Sept 8, 1995. Vol. 270, No. 36. p. 20968-20977
Publisher: Bethesda, Md. : American Society for Biochemistry and Molecular Biology.
CODEN: JBCHA3; ISSN: 0021-9258
NOTE: Includes references
PUB. COUNTRY: Maryland; United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB The bacterial gene nodE is the key determinant of host **specificity** in the Rhizobium leguminosarum-legume symbiosis and has been proposed to determine unique polyunsaturated fatty acyl moieties in chitolipooligosaccharides (CLOS) made by the bacterial symbiont. We evaluated node function by examining CLOS structures made by wild-type R. leguminosarum bv. trifolii ANU843, an isogenic nodE::Tn5 **mutant**, and a recombinant strain containing multiple copies of the pSym nod region of ANU843. 1H-NMR, electrospray ionization mass spectrometry, fast atom bombardment mass spectrometry, flame ionization detection-gas chromatography, gas chromatography/mass spectrometry, and high performance liquid chromatography/UV photodiode array analyses revealed that these bacterial strains made the same spectrum of CLOS species. We also found that ions in the mass spectra which were originally assigned to node-dependent CLOS species containing unique polyunsaturated fatty acids (Spaink, H. P., Bloemberg, G. V., van Brussel, A. A. N., Lugtenberg, B. J. J., van der Drift, K. M. G. M., Haverkamp, J., and Thomas-Oates, J. E. (1995) Mol. Plant-Microbe Interact. 8, 155-164) were actually due to

sodium adducts of the major nodE-independent CLOS species. No evidence for node-dependent CLOSs was found for these strains. These results indicate a need to revise the current model to explain how node determines host range in the R. leguminosarum-legume symbiosis.

L7 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:132500 CAPLUS
DOCUMENT NUMBER: 128:241603
TITLE: The loading domain of the erythromycin polyketide synthase is not essential for erythromycin biosynthesis in Saccharopolyspora erythraea
AUTHOR(S): Pereda, Ana; Summers, Richard G.; Stassi, Diane L.; Ruan, Xiaoan; Katz, Leonard
CORPORATE SOURCE: Antibacterial Discovery Research, Abbott Laboratories, Abbott Park, IL, 60064, USA
SOURCE: Microbiology (Reading, U. K.) (1998), 144(2), 543-553
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 6-Deoxyerythronolide B synthase (DEBS) is a large multifunctional enzyme that catalyzes the biosynthesis of the erythromycin polyketide aglycon. DEBS is organized into six modules, each contg. the enzymic domains required for a single condensation of carboxylic acid residues which make up the growing polyketide chain. Module 1 is preceded by loading acyltransferase (AT-L) and acyl carrier protein (ACP-L) domains, hypothesized to initiate polyketide chain growth with a propionate-derived moiety. Using recombinant DNA technol. several **mutant** strains of Saccharopolyspora erythraea were constructed that lack the initial AT-L domain or that lack both the AT-L and ACP-L domains. These strains were still able to produce erythromycin, although at much lower levels than that produced by the wild-type strain. In addn., the AT-L domain expressed as a monofunctional enzyme was able to complement the deletion of this domain from the PKS, resulting in increased levels of erythromycin prodn. These findings indicate that neither the initial AT-L nor the ACP-L domains are required to initiate erythromycin biosynthesis; however, without these domains the efficiency of erythromycin biosynthesis is decreased significantly. It is proposed that in these **mutants** the first step in erythromycin biosynthesis is the charging of KS1 with propionate directly from propionyl-CoA.

L7 ANSWER 16 OF 17 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:569012 CAPLUS
DOCUMENT NUMBER: 123:50757
TITLE: Daunorubicin type II polyketide synthase enzymes DpsA and DpsB determine neither the choice of starter unit nor the cyclization pattern of aromatic polyketides
AUTHOR(S): Meurer, G.; Hutchinson, C. R.
CORPORATE SOURCE: School of Pharmacy, University of Wisconsin, Madison, WI, 53706, USA
SOURCE: J. Am. Chem. Soc. (1995), 117(21), 5899-900
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To investigate the ability of different polyketide synthases to use diverse starter units and to fold and cyclize the derived linear polybetaketone intermediates in specific ways, the tcmK .beta.-**ketoacyl synthase** and tcmL chain length detg. factor genes from the tetracenomycin polyketide synthase were replaced with their dpsA and dpsB homologs from the polyketide synthase-encoding genes of daunorubicin biosynthesis. Expression of 3 such sets of polyketide synthase genes in either Streptomyces lividans 1326 or the Streptomyces glaucescens WMH1077 **mutant** strain in which the tetracenomycin polyketide synthase genes are not expressed revealed that compared with the normal tetracenomycin polyketide synthase consisting of the TcmJKLMN proteins, the hybrid polyketide synthase contg. the DpsAB/TcmMN proteins did not produce significant amts. of known compds. The set of TcmJ/DpsAB/TcmMN proteins produced prominent amts. of tetracenomycin F2 (I), an early intermediate of tetracenomycin C biosynthesis, and the TcmJ/DpsAB/TcmM proteins produced equal amts. of the known metabolites SEK15 and SEK15b plus traces of I and other unknown compds. Thus, in absence of proteins specifying the normal propionyl-CoA starter unit, the daunorubicin polyketide synthase has a relaxed **specificity** for acetyl-CoA as the primer of polyketide biosynthesis. Furthermore, because the polyketide precursor of I is cyclized at C-9/C-14, whereas the corresponding precursor of daunorubicin is cyclized at C-7/C-12, folding and cyclization of the nascent polyketide is detd. by the polyketide synthase cyclase provided (TcmN) rather than by DpsA and DpsB (or TcmK and TcmL) proteins.

L7 ANSWER 17 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:364397 BIOSIS

DOCUMENT NUMBER: PREV199497377397
TITLE: Differential activities and intramolecular location of fatty acid synthase and 6-methylsalicylic acid synthase component enzymes.
AUTHOR(S): Schorr, Roland; Mittag, Maria; Mueller, Gerhard; Schweizer, Eckhart
CORPORATE SOURCE: Lehrstuhl Biochemie, Univ. Erlangen-Nuernberg, Staudstr. 5, D-91058 Erlangen Germany
SOURCE: Journal of Plant Physiology, (1994) Vol. 143, No. 4-5, pp. 407-415.
ISSN: 0176-1617.
DOCUMENT TYPE: General Review
LANGUAGE: English
AB By targeted in vitro **mutagenesis**, the phosphopantetheine-binding serine residue of yeast fatty acid synthase (FAS) was localized in the beta-ketoacyl reductase domain (pos. 180) of FAS subunit alpha. Attachment of phosphopantetheine to serine-180 requires interaction of this N-terminal site in the protein with a distal, C-terminal domain of the same subunit. Point **mutations** in this distal region constitute a distinct complementation group (No. VII) and prevent pantetheine-binding even to an intact serine-180. In contrast to other fas2 **mutations** mapping around serine-180, the in vitro constructed serine-180 fwdarw glycine-180 **mutation** exhibits the intragenic complementation pattern of group VI (**ketoacyl synthase**-defective) rather than of group VIII (ketoacyl reductase-defective) fas-**mutants**. Thus, this **mutation** does not conform with the generally observed clustering of isofunctional fas-**mutations** within the same domain. Using stereospecifically tritiated NADPH it was shown that the beta-ketoacyl reductases of both, FAS and 6-methylsalicylic acid synthase (MSAS), exert the same **specificity** for the 4-H-Si atom of NADPH. However, the two reductases differ with respect to their ketoacyl substrate specificities: while the FAS enzyme reduces both, acetoacetyl- and triacetic acid ester model substrates, the purified MSAS is specific for the triacetyl derivative. The affinities of MSAS (K-M = 1.4 x 10-4 M) and FAS (K-M = 8.0 x 10-4 M) for triacetic acid ethyl ester were determined. Reduction of FAS-bound acetoacetate prevents it from condensation with another malonate to triacetic acid. Reduction of FAS- or MSAS-bound triacetic acid competes with its lactonization and subsequent release from the enzyme. The formation of triacetolactone is therefore always a side-reaction of 6-MSA biosynthesis. With purified MSAS, reduction of triacetic acid ethyl ester but not the subsequent step, i.e. dehydration of the 3-hydroxy acid to 5-oxo-2,3-hexenic acid ethyl ester, could be experimentally verified. It is therefore concluded that the dehydration step occurs at the tetracetic rather than at the triacetic acid level. Cyclization and aromatization of the corresponding intermediate to 6-MSA would then occur at the same time. Enzyme-bound 6-MSA is finally released from the enzyme hydrolytically rather than as a coenzyme A thioester. Hence, the two polyketide synthases, MSAS and FAS, containing a very similar set of catalytic domains produce their specific end products obviously as a consequence of distinctly different substrate specificities of their beta-ketoacyl reductase, dehydratase and terminal acyl transferase component enzymes.